

12-15-99

A

Express Mail Label No. TB553893263US
Practitioner's Docket No. 45753-DIV2
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Box Patent Application
Assistant Commissioner for Patents
Washington, D.C. 20231

NEW APPLICATION TRANSMITTAL

Transmitted herewith for filing is the patent application of

Inventor(s): Suji HINUMA, Yasuaki ITO, Ryo FUJII

WARNING: 37 CFR 1.41(a)(1) points out

"(a) A patent is applied for in the name or names of the actual inventor or inventors

(1) The inventorship of a nonprovisional application is that inventorship set forth in the oath or declaration as prescribed by § 1.63, except as provided for in § 1.53(d)(4) and § 1.63(d). If an oath or declaration as prescribed by § 1.63 is not filed during the pendency of a nonprovisional application, the inventorship is that inventorship set forth in the application papers filed pursuant to § 1.53(b), unless a petition under this paragraph accompanied by the fee set forth in § 1.17(i) is filed supplying or changing the name or names of the inventor or inventors."

For (title): G PROTEIN COUPLED RECEPTOR PROTEIN PRODUCTION, AND USE THEREOF

CERTIFICATION UNDER 37 C.F.R. 1.10*

(Express Mail label number is **mandatory**)
(Express Mail certification is optional)

I hereby certify that this correspondence and the documents referred to as attached therein are being deposited with the United States Postal Service on this date December 14, 1999, in an envelope as "Express Mail Post Office to Addressee," mailing Label Number TB553893263US, addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

Donna M. Tomaso

(type or print name of person mailing paper)

Signature of person mailing paper

WARNING: Certificate of mailing (first class) or facsimile transmission procedures of 37 C.F.R. 1.8 cannot be used to obtain a date of mailing or transmission for this correspondence

***WARNING:** Each paper or fee filed by "Express Mail" **must** have the number of the "Express Mail" mailing label placed thereon prior to mailing 37 C.F.R. 1.10(b)
"Since the filing of correspondence under § 1.10 without the Express Mail mailing label thereon is an oversight that can be avoided by the exercise of reasonable care, requests for waiver of this requirement will **not** be granted on petition." Notice of Oct. 24, 1996, 60 Fed. Reg. 56,439, at 56,442

1. Type of Application

This new application is for a(n)

(check one applicable item below)

☐ Original (nonprovisional)

☐ Design

☐ Plant

WARNING: *Do not use this transmittal for a completion in the U S of an International Application under 35 U S C 371(c)(4), unless the International Application is being filed as a divisional, continuation or continuation-in-part application*

WARNING: *Do not use this transmittal for the filing of a provisional application*

NOTE: *If one of the following 3 items apply, then complete and attach ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF A PRIOR U S APPLICATION CLAIMED and a NOTIFICATION IN PARENT APPLICATION OF THE FILING OF THIS CONTINUATION APPLICATION*

☒ Divisional.

☐ Continuation.

☐ Continuation-in-part (C-I-P).

2. Benefit of Prior U.S. Application(s) (35 U.S.C. 119(e), 120, or 121)

NOTE. *A nonprovisional application may claim an invention disclosed in one or more prior filed copending nonprovisional applications or copending international applications designating the United States of America. In order for a nonprovisional application to claim the benefit of a prior filed copending nonprovisional application or copending international application designating the United States of America, each prior application must name as an inventor at least one inventor named in the later filed nonprovisional application and disclose the named inventor's invention claimed in at least one claim of the later filed nonprovisional application in the manner provided by the first paragraph of 35 U S C 112. Each prior application must also be*

(i) An international application entitled to a filing date in accordance with PCT Article 11 and designating the United States of America, or

(ii) Complete as set forth in § 1 51(b), or

(iii) Entitled to a filing date as set forth in § 1 53(b) or § 1 53(d) and include the basic filing fee set forth in § 1 16, or

(iv) Entitled to a filing date as set forth in § 1 53(b) and have paid therein the processing and retention fee set forth in § 1 21(l) within the time period set forth in § 1 53(f)

37 CFR 1 78(a)(1)

NOTE *If the new application being transmitted is a divisional, continuation or a continuation-in-part of a parent case, or where the parent case is an International Application which designated the U S , or benefit of a prior provisional*

application is claimed, then check the following item and complete and attach ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U S APPLICATION(S) CLAIMED

WARNING: If an application claims the benefit of the filing date of an earlier filed application under 35 U S C 120, 121 or 365(c), the 20-year term of that application will be based upon the filing date of the earliest U S application that the application makes reference to under 35 U S C 120, 121 or 365(c) (35 U.S.C. 154(a)(2) does not take into account, for the determination of the patent term, any application on which priority is claimed under 35 U S C 119, 365(a) or 365(b)) For a c-i-p application, applicant should review whether any claim in the patent that will issue is supported by an earlier application and, if not, the applicant should consider canceling the reference to the earlier filed application The term of a patent is not based on a claim-by-claim approach See Notice of April 14, 1995, 60 Fed Reg 20,195, at 20,205

WARNING: When the last day of pendency of a provisional application falls on a Saturday, Sunday, or Federal holiday within the District of Columbia, any nonprovisional application claiming benefit of the provisional application **must** be filed prior to the Saturday, Sunday, or Federal holiday within the District of Columbia. See 37 C.F.R. § 1.78(a)(3)

☒ [X] The new application being transmitted claims the benefit of prior U.S. application(s).
Enclosed are ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

3. Papers Enclosed

A. Required for Filing Date under 37 C.F.R. 1.53(b) (Regular) or 37 C.F.R. 1.153 (Design) Application

270 Pages of Specification
6 Pages of Claims
79 Sheets of Drawing

☒ [X] Formal
☐ [] Informal

B. Other Papers Enclosed

1 Pages of Abstract
 Other

WARNING: **DO NOT** submit original drawings A high quality copy of the drawings should be supplied when filing a patent application The drawings that are submitted to the Office must be on strong, white, smooth, and non-shiny paper and meet the standards according to § 1.84 If corrections to the drawings are necessary, they should be made to the original drawing and a high-quality copy of the corrected original drawing then submitted to the Office Only one copy is required or desired For comments on proposed then-new 37 C F R 1.84, see Notice of March 9, 1988 (1990 O G 57-62)

NOTE: "Identifying indicia, if provided, should include the application number or the title of the invention, inventor's name, docket number (if any), and the name and telephone number of a person to call if the Office is unable to match the drawings to the proper application This information should be placed on the back of each sheet of drawing a minimum distance of 1.5 cm (5/8 inch) down from the top of the page " 37 C F R 1.84(c))

(complete the following, if applicable)

- ☐ The enclosed drawing(s) are photograph(s), and there is also attached a "PETITION TO ACCEPT PHOTOGRAPH(S) AS DRAWING(S)." 37 C.F.R. 1.84(b).

4. Additional Papers Enclosed

- ☒ Preliminary Amendment
☒ Information Disclosure Statement (37 C.F.R. 1.98)
☒ Form PTO-1449 (PTO/SB/08A and 08B)
☐ Citations
☐ Declaration of Biological Deposit
☒ Submission of "Sequence Listing," computer readable copy and/or amendment pertaining thereto for biotechnology invention containing nucleotide and/or amino acid sequence.
☐ Authorization of Attorney(s) to Accept and Follow Instructions from Representative
☐ Special Comments
☐ Other:

5. Declaration or Oath

NOTE: *A newly executed declaration is not required in a continuation or divisional application provided the prior nonprovisional application contained a declaration as required, the application being filed is by all or fewer than all the inventors named in the prior application, there is no new matter in the application being filed, and a copy of the executed declaration filed in the prior application (showing the signature or an indication thereon that it was signed) is submitted. The copy must be accompanied by a statement requesting deletion of the names of person(s) who are not inventors of the application being filed. If the declaration in the prior application was filed under § 1.47 then a copy of that declaration must be filed accompanied by a copy of the decision granting § 1.47 status or, if a nonsigning person under § 1.47 has subsequently joined in a prior application, then a copy of the subsequently executed declaration must be filed. See 37 CFR 1.63(d).*

NOTE: *A declaration filed to complete an application must be executed, identify the specification to which it is directed, identify each inventor by full name, including the family name, and at least one given name without abbreviation together with any other given name or initial, and the residence, post office address and country of citizenship of each inventor and state whether the inventor is a sole or joint inventor. 37 CFR 1.63(a)(1)-(4).*

- ☐ Enclosed

Executed by

(check **all** applicable boxes)

- ☐ inventor(s).
☐ legal representative of inventor(s). 37 CFR 1.42 or 1.43.
☐ joint inventor or person showing a proprietary interest on behalf of inventor who refused to sign or cannot be reached.
☐ This is the petition required by 37 CFR 1.47 and the statement required by 37 CFR 1.47 is also attached. See item 13 below for fee.

- ☒ Not Enclosed.

NOTE: *Where the filing is a completion in the U.S. of an International Application, or where the completion of the U.S. application contains subject matter in addition to the International Application, the application may be treated as a continuation or continuation-in-part, as the case may be, utilizing ADDED PAGE FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION CLAIMED*

☒ Application is made by a person authorized under 37 C.F.R. 1.41(c) on behalf of all the above named inventor(s).

(The declaration or oath, along with the surcharge required by 37 CFR 1.16(e), can be filed subsequently).

NOTE It is important that all the correct inventor(s) are named for filing under 37 CFR 1.41(c) and 1.53(b)

☐ Showing that the filing is authorized.
(not required unless called into question. 37 CFR 1.41(d))

6. Inventorship Statement

WARNING: If the named inventors are each not the inventors of all the claims an explanation, including the ownership of the various claims at the time the last claimed invention was made, should be submitted

The inventorship for all the claims in this application are:

☐ The same.

or

☐ Not the same. An explanation, including the ownership of the various claims at the time the last claimed invention was made,

☐ is submitted.

☐ will be submitted.

7. Language

NOTE: An application including a signed oath or declaration may be filed in a language other than English. An English translation of the non-English language application and the processing fee of \$130.00 required by 37 CFR 1.17(k) is required to be filed with the application, or within such time as may be set by the Office. 37 CFR 1.52(d)

☒ English

☐ Non-English

☐ The attached translation includes a statement that the translation is accurate. 37 C.F.R. 1.52(d).

8. Assignment

☒ An assignment of the invention to Takeda Chemical Industries, Ltd.,
1-1, Doshomachi, 4-chome, Chuo-ku,
Osaka-shi, Osaka 541 Japan

☐ is attached. A separate ☐ "COVER SHEET FOR ASSIGNMENT (DOCUMENT) ACCOMPANYING NEW PATENT APPLICATION" or ☐ FORM PTO 1595 is also attached.

☐ was filed in the parent application

☒ will follow.

NOTE "If an assignment is submitted with a new application, send two separate letters-one for the application and one for the assignment" Notice of May 4, 1990 (1114 O G 77-78)

WARNING: A newly executed "STATEMENT UNDER 37 CFR 3 73(b)" must be filed when a continuation-in-part application is filed by an assignee Notice of April 30, 1993, 1150 O G 62-64

9. Certified Copy

Certified copy(ies) of application(s)

	<u>Country</u>	<u>Appln. No.</u>	<u>Filed</u>
(1)	Japan	7-074314	March 31, 1995
(2)	Japan	6-189272	August 11, 1994
(3)	Japan	6-189273	August 11, 1994
(4)	Japan	6-189274	August 11, 1994
(5)	Japan	6-236356	September 30, 1994
(6)	Japan	6-236357	September 30, 1994
(7)	Japan	6-270017	November 2, 1994
(8)	Japan	6-326611	December 28, 1994
(9)	Japan	7-007177	January 20, 1995
(10)	Japan	7-057186	March 16, 1995
(11)	Japan	7-093989	April 19, 1995

from which priority is claimed

☒ is enclosed (1).
☒ was filed (2-11).
☐ will follow.

NOTE: The foreign application forming the basis for the claim for priority must be referred to in the oath or declaration 37 CFR 1 55(a) and 1 63

NOTE: This item is for any foreign priority for which the application being filed directly relates. If any parent U.S. application or International Application from which this application claims benefit under 35 U S C 120 is itself entitled to priority from a prior foreign application, then complete item 18 on the ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U S. APPLICATION(S) CLAIMED

10. Fee Calculation (37 C.F.R. 1.16)

A. ☒ Regular application

CLAIMS AS FILED

Claims	Number Filed	Basic Fee Allowance	Number Extra	Rate	Basic Fee 37 C.F.R. 1.16(a) \$760.00
Total Claims (37 CFR 1.16(c))	8	- 20 =	0	x \$ 18.00	
Independent Claims	1	- 3 =	0	x \$78.00	

(37 CFR 1.16(b))

Multiple Dependent
Claim(s), if any
(37 CFR 1.16(d))

+ \$260.00

- ☐ Amendment cancelling extra claims is enclosed.
☐ Amendment deleting multiple-dependencies is enclosed.
☐ Fee for extra claims is not being paid at this time.

NOTE: *If the fees for extra claims are not paid on filing they must be paid or the claims cancelled by amendment, prior to the expiration of the time period set for response by the Patent and Trademark Office in any notice of fee deficiency 37 CFR 1.16(d)*

Filing Fee Calculation \$ 760.00

B. ☐ Design application
(\$330.00—37 CFR 1.16(f))

Filing Fee Calculation \$

C. ☐ Plant application
(\$540.00—37 CFR 1.16(g))

Filing Fee Calculation \$

11. Small Entity Statement(s)

- ☐ Statement(s) that this is a filing by a small entity under 37 CFR 1.9 and 1.27 is (are) attached.

WARNING: *"Status as a small entity must be specifically established in each application or patent in which the status is available and desired. Status as a small entity in one application or patent does not affect any other application or patent, including applications or patents which are directly or indirectly dependent upon the application or patent in which the status has been established. The refiling of an application under § 1.53 as a continuation, division, or continuation-in-part (including a continued prosecution application under § 1.53(d)), or the filing of a reissue application requires a new determination as to continued entitlement to small entity status for the continuing or reissue application. A nonprovisional application claiming benefit under 35 U.S.C. 119(e), 120, 121, or 365(c) of a prior application, or a reissue application may rely on a statement filed in the prior application or in the patent if the nonprovisional application or the reissue application includes a reference to the statement in the prior application or in the patent or includes a copy of the statement in the prior application or in the patent and status as a small entity is still proper and desired. The payment of the small entity basic statutory filing fee will be treated as such a reference for purposes of this section." 37 CFR 1.28(a)(2)*

(complete the following, if applicable)

- ☐ Status as a small entity was claimed in prior application _____, filed on _____ from which benefit is being claimed for this application under:

35 U.S.C. § ☐ 119(e),
☐ 120,
☐ 121,

☐ 365(c),

and which status as a small entity is still proper and desired.

☐ A copy of the statement in the prior application is included.

Filing Fee Calculation (50% of **A**, **B** or **C** above) \$ _____

NOTE: Any excess of the full fee paid will be refunded if a small entity status is established refund request are filed within 2 months of the date of timely payment of a full fee The two-month period is not extendable under § 1.136 37 CFR 1.28(a).

12. Request for International-Type Search (37 C.F.R. 1.104(d))

(complete, if applicable)

☐ Please prepare an international-type search report for this application at the time when national examination on the merits takes place.

13. Fee Payment Being Made at This Time

☒ Not Enclosed

☒ No filing fee is to be paid at this time.

(This and the surcharge required by 37 C.F.R. 1.16(e) can be paid subsequently.)

☐ Enclosed

☐ Filing fee \$ _____

☐ Recording assignment
(\$40.00; 37 C.F.R. 1.21(h))
(See attached "COVER SHEET FOR
ASSIGNMENT ACCOMPANYING NEW
APPLICATION.") \$ _____

☐ Petition fee for filing by other than
all the inventors or person on behalf
of the inventor where inventor
refused to sign or cannot be reached
(\$130.00; 37 C.F.R. 1.47 and 1.17(i)) \$ _____

☐ For processing an application with a
specification in a non-English language
(\$130.00; 37 C.F.R. 1.52(d) and 1.17(k)) \$ _____

☐ Processing and retention fee
(\$130.00; 37 C.F.R. 1.53(d) and 1.21(l)) \$ _____

☐ Fee for international-type search report
(\$40.00; 37 C.F.R. 1.21(e)) \$_____

NOTE: 37 CFR 1.21(l) establishes a fee for processing and retaining any application that is abandoned for failing to complete the application pursuant to 37 CFR 1.53(f) and this, as well as the changes to 37 CFR 1.53 and 1.78(a)(1), indicate that in order to obtain the benefit of a prior U.S. application, either the basic filing fee must be paid, or the processing and retention fee of § 1.21(l) must be paid, within 1 year from notification under § 53(f)

Total Fees Enclosed \$_____

14. Method of Payment of Fees

- ☐ Check in the amount of \$_____.
- ☐ Charge Account No. _____ in the amount of \$_____.
A duplicate of this transmittal is attached.

NOTE: Fees should be itemized in such a manner that it is clear for which purpose the fees are paid 37 CFR 1.22(b)

15. Authorization to Charge Additional Fees

WARNING: If no fees are to be paid on filing, the following items should not be completed

WARNING: Accurately count claims, especially multiple dependent claims, to avoid unexpected high charges, if extra claim charges are authorized

- ☐ The Commissioner is hereby authorized to charge the following additional fees by this paper and during the entire pendency of this application to Account No._____.
- ☐ 37 C.F.R. 1.16(a), (f) or (g) (filing fees)
- ☐ 37 C.F.R. 1.16(b), (c) and (d) (presentation of extra claims)

NOTE: Because additional fees for excess or multiple dependent claims not paid on filing or on later presentation must only be paid or these claims cancelled by amendment prior to the expiration of the time period set for response by the PTO in any notice of fee deficiency (37 CFR 1.16(d)), it might be best not to authorize the PTO to charge additional claim fees, except possibly when dealing with amendments after final action

- ☐ 37 C.F.R. 1.16(e) (surcharge for filing the basic filing fee and/or declaration on a date later than the filing date of the application)
- ☐ 37 CFR 1.17(a)(1)-(5) (extension fees pursuant to § 1.136(a).
- ☐ 37 C.F.R. 1.17 (application processing fees)

NOTE "A written request may be submitted in an application that is an authorization to treat any concurrent or future reply, requiring a petition for an extension of time under this paragraph for its timely submission, as incorporating a petition for extension of time for the appropriate length of time. An authorization to charge all required fees, fees under § 1.17, or all required extension of time fees will be treated as a constructive petition for an extension of time in any concurrent or future reply requiring a petition for an extension of time under this paragraph for its timely submission. Submission of the fee set forth in § 1.17(a) will also be treated as a constructive petition for an extension of time in any concurrent reply requiring a petition for an extension of time under this paragraph for its timely submission." 37 CFR 1.136(a)(3)

☐ 37 C.F.R. 1.18 (issue fee at or before mailing of Notice of Allowance, pursuant to 37 C.F.R. 1.311(b))

NOTE: Where an authorization to charge the issue fee to a deposit account has been filed before the mailing of a Notice of Allowance, the issue fee will be automatically charged to the deposit account at the time of mailing the notice of allowance. 37 CFR 1.311(b)).

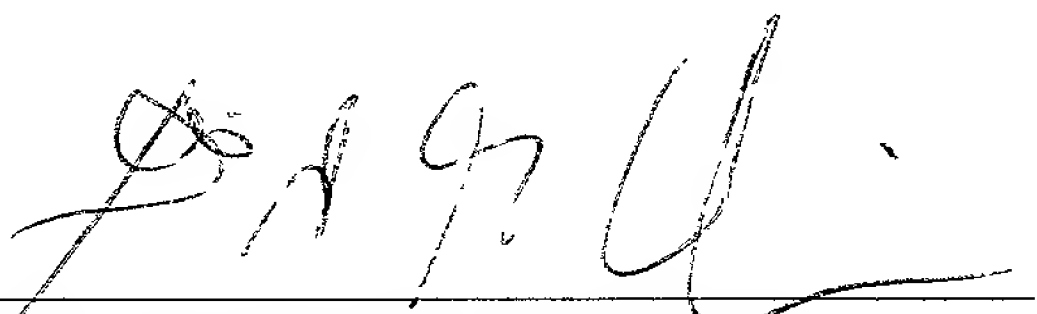
NOTE: 37 CFR 1.28(b) requires "Notification of any change in status resulting in loss of entitlement to small entity status must be filed in the application prior to paying, or at the time of paying, issue fee." From the wording of 37 CFR 1.28(b), (a) notification of change of status must be made even if the fee is paid as "other than a small entity" and (b) no notification is required if the change is to another small entity.

16. Instructions as to Overpayment

NOTE: "... Amounts of twenty-five dollars or less will not be returned unless specifically requested within a reasonable time, nor will the payer be notified of such amounts, amounts over twenty-five dollars may be returned by check or, if requested, by credit to a deposit account." 37 CFR 1.26(a)

☒ Credit Account No. 04-1105.

☐ Refund



SIGNATURE OF PRACTITIONER

Reg. No. 27,026

David G. Conlin
(type or print name of practitioner)

Tel. No.: (617) 523-3400

Dike, Bronstein, Roberts & Cushman, LLP
130 Water Street
P.O. Address

Customer No.: 21874

Boston, MA 02109

☒ **Incorporation by reference of added pages**

(check the following item if the application in this transmittal claims the benefit of prior U.S. application(s) (including an international application entering the U.S. stage as a continuation, divisional or C-I-P application) and complete and attach the ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED)

☒ Plus Added Pages for New Application Transmittal Where Benefit of Prior U.S. Application(s) Claimed

Number of pages added 5

☒ Plus Added Pages for Papers Referred to in Item 4 Above

Number of pages added 20

☐ Plus added pages deleting names of inventor(s) named on prior application(s) who is/are no longer inventor(s) of the subject matter claimed in this application.

Number of pages added _____

☒ Plus "Assignment Cover Letter Accompanying New Application"

Number of pages added 3

☐ **Statement Where No Further Pages Added**

(if no further pages form a part of this Transmittal, then end this Transmittal with this page and check the following item)

☐ This transmittal ends with this page.

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PATENT

ADDED PAGES FOR APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED

NOTE: See 37 CFR 1.78.

17. Relate Back

WARNING: *If an application claims the benefit of the filing date of an earlier filed application under 35 U.S.C. 120, 121 or 365(c), the 20-year term of that application will be based upon the filing date of the earliest U.S. application that the application makes reference to under 35 U.S.C. 120, 121 or 365(c). (35 U.S.C. 154(a)(2) does not take into account, for the determination of the patent term, any application on which priority is claimed under 35 U.S.C. 119, 365(a) or 365(b).) For a c-i-p application, applicant should review whether any claim in the patent that will issue is supported by an earlier application and, if not, the applicant should consider canceling the reference to the earlier filed application. The term of a patent is not based on a claim-by-claim approach. See Notice of April 14, 1995, 60 Fed. Reg. 20,195, at 20,205.*

(complete the following, if applicable)

☒ Amend the specification by inserting, before the first line, the following sentence:

A. 35 U.S.C. 119(e)

NOTE: *"Any nonprovisional application claiming the benefit of one or more prior filed copending provisional applications must contain or be amended to contain in the first sentence of the specification following the title a reference to each such prior provisional application, identifying it as a provisional application, and including the provisional application number (consisting of series code and serial number)." 37 C.F.R. § 1.78(a)(4).*

☐ "This application claims the benefit of U.S. Provisional Application(s) No(s).:

APPLICATION NO(S):

FILING DATE

	"
	"
/	"

B. 35 U.S.C. 120, 121 and 365(c)

NOTE: *"Except for a continued prosecution application filed under § 1.53(d), any nonprovisional application claiming the benefit of one or more prior filed copending nonprovisional applications or international applications designating the United States of America must contain or be amended to contain in the first sentence of the specification following the title a reference to each such prior application, identifying it by application number (consisting of the series code and serial number) or international application number and international filing date and indicating the relationship of the applications . . . Cross-references to other related applications may be made when appropriate " (See § 1.14(a)). 37 C.F.R. § 1.78(a)(2).*

☒ "This application is a

☐ continuation

☐ continuation-in-part

☒ divisional

of copending application(s)

☒ application number 09/038,572, filed 3/11/98, which is a divisional of U.S.S.N. 08/513,974, filed 9/14/95, which is a continuation of International Application No. PCT/JP95/01599, filed 8/10/95, which designated the U.S.”

☐ International Application _____ filed on _____ and which designated the U.S.”

NOTE: The proper reference to a prior filed PCT application that entered the U.S. national phase is the U.S. serial number and the filing date of the PCT application that designated the U.S.

NOTE: (1) Where the application being transmitted adds subject matter to the International Application, then the filing can be as a continuation-in-part or (2) if it is desired to do so for other reasons then the filing can be as a continuation.

NOTE: The deadline for entering the national phase in the U.S. for an international application was clarified in the Notice of April 28, 1987 (1079 O.G. 32 to 46) as follows:

“The Patent and Trademark Office considers the International application to be pending until the 22nd month from the priority date if the United States has been designated and no Demand for International Preliminary Examination has been filed prior to the expiration of the 19th month from the priority date and until the 32nd month from the priority date if a Demand for International Preliminary Examination which elected the United States of America has been filed prior to the expiration of the 19th month from the priority date, provided that a copy of the international application has been communicated to the Patent and Trademark Office within the 20 or 30 month period respectively. If a copy of the international application has not been communicated to the Patent and Trademark Office within the 20 or 30 month period respectively, the international application becomes abandoned as to the United States 20 or 30 months from the priority date respectively. These periods have been placed in the rules as paragraph (h) of § 1.494 and paragraph (i) of § 1.495. A continuing application under 35 U.S.C. 365(c) and 120 may be filed anytime during the pendency of the international application ”

☐ “The nonprovisional application designated above, namely application _____/_____, filed _____, claims the benefit of U.S. Provisional Application(s) No(s).:

APPLICATION NO(S).:

FILING DATE

_____/_____
_____/_____
_____/_____

_____,
_____,
_____”

☐ Where more than one reference is made above please combine all references into one sentence.

18. Relate Back—35 U.S.C. 119 Priority Claim for Prior Application

The prior U.S. application(s), including any prior International Application designating the U.S., identified above in item 17B, in turn itself claim(s) foreign priority(ies) as follows:

Country	Appln. no.	Filed
(1) Japan	7-074314	March 31, 1995
(2) Japan	6-189272	August 11, 1994
(3) Japan	6-189273	August 11, 1994
(4) Japan	6-189274	August 11, 1994
(5) Japan	6-236356	September 30, 1994
(6) Japan	6-236357	September 30, 1994
(7) Japan	6-270017	November 2, 1994
(8) Japan	6-326611	December 28, 1994
(9) Japan	7-007177	January 20, 1995
(10) Japan	7-057186	March 16, 1995
(11) Japan	7-093989	April 19, 1995

The certified copy(ies) has (have)

☒ been filed on _____, in prior application PCT/JP95/01599, which was filed on
8/10/95 (2-11).

☒ is (are) attached (1).

WARNING: *The certified copy of the priority application that may have been communicated to the PTO by the International Bureau may not be relied on without any need to file a certified copy of the priority application in the continuing application. This is so because the certified copy of the priority application communicated by the International Bureau is placed in a folder and is not assigned a U.S. serial number unless the national stage is entered. Such folders are disposed of if the national stage is not entered. Therefore, such certified copies may not be available if needed later in the prosecution of a continuing application. An alternative would be to physically remove the priority documents from the folders and transfer them to the continuing application. The resources required to request transfer, retrieve the folders, make suitable record notations, transfer the certified copies, enter and make a record of such copies in the Continuing Application are substantial. Accordingly, the priority documents in folders of international applications that have not entered the national stage may not be relied on. Notice of April 28, 1987 (1079 O.G. 32 to 46)*

19. Maintenance of Copendency of Prior Application

NOTE: *The PTO finds it useful if a copy of the petition filed in the prior application extending the term for response is filed with the papers constituting the filing of the continuation application. Notice of November 5, 1985 (1060 O.G. 27).*

A. ☐ Extension of time in prior application

*(This item **must** be completed and the papers filed in the prior application, if the period set in the prior application has run.)*

☐ A petition, fee and response extends the term in the pending **prior** application until _____

☐ A **copy** of the petition filed in prior application is attached.

B. ☐ Conditional Petition for Extension of Time in Prior Application

(complete this item, if previous item not applicable)

☐ A conditional petition for extension of time is being filed in the pending **prior** application.

☐ A **copy** of the conditional petition filed in the prior application is attached.

20. Further Inventorship Statement Where Benefit of Prior Application(s) Claimed

(complete applicable item (a), (b) and/or (c) below)

(a) ☐ This application discloses and claims only subject matter disclosed in the prior application whose particulars are set out above and the inventor(s) in this application are

☐ the same.

☐ less than those named in the prior application. It is requested that the following inventor(s) identified for the prior application be deleted:

(type name(s) of inventor(s) to be deleted)

(b) ☐ This application discloses and claims additional disclosure by amendment and a new declaration or oath is being filed. With respect to the prior application, the inventor(s) in this application are

☐ the same.

☐ the following additional inventor(s) have been added:

(type name(s) of inventor(s) to be deleted)

(c) ☐ The inventorship for all the claims in this application are

☐ the same.

☐ not the same. An explanation, including the ownership of the various claims at the time the last claimed invention was made

☐ is submitted.

☐ will be submitted.

21. Abandonment of Prior Application (if applicable)

- ☐ Please abandon the prior application at a time while the prior application is pending, or when the petition for extension of time or to revive in that application is granted, and when this application is granted a filing date, so as to make this application copending with said prior application.

NOTE: According to the Notice of May 13, 1983 (103, TMOG 6-7), the filing of a continuation or continuation-in-part application is a proper response with respect to a petition for extension of time or a petition to revive and should include the express abandonment of the prior application conditioned upon the granting of the petition and the granting of a filing date to the continuing application.

22. Petition for Suspension of Prosecution for the Time Necessary to File an Amendment

WARNING: "The claims of a new application may be finally rejected in the first Office action in those situations where (1) the new application is a continuing application of, or a substitute for, an earlier application, and (2) all the claims of the new application (a) are drawn to the same invention claimed in the earlier application, and (b) would have been properly finally rejected on the grounds of art of record in the next Office action if they had been entered in the earlier application." MPEP, § 706.07(b).

NOTE: Where it is possible that the claims on file will give rise to a first action final for this continuation application and for some reason an amendment cannot be filed promptly (e.g., experimental data is being gathered) it may be desirable to file a petition for suspension of prosecution for the time necessary.

(check the next item, if applicable)

- ☐ There is provided herewith a Petition To Suspend Prosecution for the Time Necessary to File An Amendment (New Application Filed Concurrently)

23. Small Entity (37 CFR § 1.28(a))

- ☐ Applicant has established small entity status by the filing of a statement in parent application No. _____.
- ☐ A copy of the statement previously filed is included.

WARNING: See 37 CFR § 1.28(a).

24. NOTIFICATION IN PARENT APPLICATION OF THIS FILING

- ☐ A notification of the filing of this
(check one of the following)
- ☐ continuation
☐ continuation-in-part
☐ divisional

is being filed in the parent application, from which this application claims priority under 35 U.S.C. § 120.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application: S. Hinuma, et al.

Serial No.: Not Yet Assigned EXAMINER: Not Yet Assigned
Filed: Herewith ART UNIT: Not Yet Assigned
For: G PROTEIN COUPLED RECEPTOR PROTEIN, PRODUCTION, AND
USE THEREOF

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

CERTIFICATE OF MAILING

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the United States Postal Service with sufficient postage as Express Mail (Express Mail Label No. TB553893263US) in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231 on December 14, 1999.

By: _____

Donna M. Tomasco

PRELIMINARY AMENDMENT

Please amend the above-identified application filed herewith as follows:

In the Specification:

Page 61, line 18, after "genes" insert --(SEQ ID NOS: 62-75)--;
line 23, after "genes" insert --(SEQ ID NOS: 76-91)--;
line 30, after "genes" insert --(SEQ ID NOS: 92-110)--.

Page 62, line 1, after “genes” insert --(SEQ ID NOS: 111-121)--;
line 9, after “genes” insert --(SEQ ID NOS: 122-142)--;
line 14, after “genes” insert --(SEQ ID NOS:143-154)--;
line 19, after “genes” insert --(SEQ ID NOS:155-171)--;
line 24, after “genes” insert --(SEQ ID NOS:172-191)--;
line 29, after “genes” insert --(SEQ ID NOS:192-204)--;
line 34, after “genes” insert --(SEQ ID NOS: 205-218)--.

Page 63, line 2, after “genes” insert --(SEQ ID NOS: 219-229)--;
line 7, after “genes” insert --(SEQ ID NOS: 230-242)--;
line 12, after “genes” insert --(SEQ ID NOS: 243-254)--;
line 17, after “genes” insert --(SEQ ID NOS: 255-274)--;
line 22, after “genes” insert --(SEQ ID NOS: 275-286)--;
line 27, after “genes’ insert --(SEQ ID NOS: 287-299)--.

Page 64, line 4, after “A58” insert --(SEQ ID NO:300)--;
line 7, after “pCRTMII” insert --(HUMSOMAT: SEQ ID NO: 301)--;
line 9, after “primer” insert -- (SEQ ID NO: 302)(HUMSOMATA: SEQ
ID NO: 303)--;
line 11, after “57-A-2” insert --(SEQ ID NO:304)--;
line 14, after “pCRTMII” insert --(HUMDRDSA:SEQ ID NO305)--;
line 17, after “B54” insert --(SEQ ID NO:306--;
line 19, after “pCRTMII” insert --(RNUO4738: SEQ ID NO:307)--;
line 20, after “sequence” insert --(SEQ ID NO:308)--;
line 24, after “sequence” insert --(SEQ ID NO:309)--;
line 27, after “sequence” insert --(SEQ ID NO:310)--;
line 31, after “sequence” insert --(SEQ ID NO:311)--.

Page 65, line 8, after "(P19P2)" insert --(SEQ ID NO:312)--;
line 11, after "(S12863" insert --(SEQ ID NO:313)--;
line 17, after "sequence" insert --(SEQ ID NO:314)--;
line 23, after "encoded" insert --(SEQ ID NO:315)--;
line 29, after "sequence" insert --(SEQ ID NO:316)--;
line 32, after "sequence" insert --(SEQ ID NO:317)--;
line 34, after "sequence" insert --(SEQ ID NO:318)--;
line 37, after "sequence" insert --(SEQ ID NO:319)--.

Page 66, line 10, after "(p63A2)" insert --(SEQ ID NO:320)--;
line 13, after "(P30731)" insert --(SEQ ID NO:321)--;
line 16, after "sequence" insert --(SEQ ID NO:322)--;
line 21, after "sequence" insert --(SEQ ID NO:323)--;
line 29, after "sequence" insert --(SEQ ID NO:324)--;
line 32, after "sequence" insert --(SEQ ID NO:325)--.

Page 67, line 3, after "p3H2-17" insert --(SEQ ID NO:326)--;
line 5, after "(P34996)" insert --(SEQ ID NO:327)--;
line 6, after "(A46226)" insert --(SEQ ID NO:328)--;
line 7, after "(JN0605)" insert --(SEQ ID NO:329)--;
line 8, after "(S28787)" insert --(SEQ ID NO:330)--;
line 9, after "sequence" insert --(SEQ ID NO:331)--;
line 12, after "sequence" insert --(SEQ ID NO:332)--;
line 22, after "p3H2-34" insert --(SEQ ID NO:333)--;
line 24, after "(JNO605)" insert --(SEQ ID NO:334)--;
line 25, after "(B41795)" insert --(SEQ ID NO:335)--;
line 26, after "(A39297)" insert --(SEQ ID NO:336)--;
line 28, after "sequence" insert --(SEQ ID NO:337)--;
line 33, after "sequence" insert --(SEQ ID NO:338)--.

Page 68, line 5, after "(pMD4)" insert --(SEQ ID NO:339)--;
line 10, after "(A35639)" insert --(SEQ ID NO:340)--;
line 14, after "sequence" insert --(SEQ ID NO:341)--;
line 17, after "thereby" insert --(SEQ ID NO:342)--;
line 24, after "(MOUSEGALRECE)" insert --(SEQ ID NO:343)--;
line 26, after "(HUMAGALAMI)" insert --(SEQ ID NO:344)--;
line 29, after "sequence" insert --(SEQ ID NO:345)--;
line 34, after "thereby" insert --(SEQ ID NO:346)--;

Page 69, line 6, after "(pMJ10)" insert --(SEQ ID NO:347)--;
line 10, after "(B42009)" insert --(SEQ ID NO:348)--;
line 11, after "(JC2014)" insert --(SEQ ID NO:349)--;
line 12, after "(A46520)" insert --(SEQ ID NO:350)--;
line 13, after "(A46525)" insert --(SEQ ID NO:351)--;
line 13, after "(S28787)" insert --(SEQ ID NO:352)--;
line 18, after "sequence" insert --(SEQ ID NO:353)--;
line 23, after "thereby" insert --(SEQ ID NO:354)--;
line 32, after "(pMH28)" insert --(SEQ ID NO:355)--;
line 36, after "(P35343)" insert --(SEQ ID NO:356)--;
line 37, after "(A41795)" insert --(SEQ ID NO:357)--;
line 37, after "(A47457)" insert --(SEQ ID NO:358)--.

Page 70, line 5, after "sequence" insert --(SEQ ID NO:359)--;
line 10, after "thereby" insert --(SEQ ID NO:360)--;
line 13, after "sequence" insert --(SEQ ID NO:359)--;
line 18, after "thereby" insert --(SEQ ID NO:360)--.

Page 71, line 1, after "(p19P2)" insert --(SEQ ID NO:361)--;
line 4, after "S12863" insert --(SEQ ID NO:362)--;
line 11, after "(pG3-2/pG1-10)" insert --(SEQ ID NO:363)--;
line 13, after "(p19P2)" insert --(SEQ ID NO:364)--;
line 22, after "sequence" insert --(SEQ ID NO:365)--;
line 25, after "sequence" insert --(SEQ ID NO:366)--;
line 28, after "(p5S38)" insert --(SEQ ID NO:369)--;
line 31, after "(p19P2)" insert --(SEQ ID NO:367)--;
line 33, after "sequence" insert --(SEQ ID NO:368)--.

Page 74, line 32, after "sequence" insert --(SEQ ID NO:370)--.

Page 75, line 1, after "sequence" insert --(SEQ ID NO:371)--;
line 6, after "(75+13CODING)" insert --(SEQ ID NO:372)--;
line 10, after "(P2UR MOUSE)" insert --(SEQ ID NO:373)--;
line 11, after "(P2YR CHICK)" insert --(SEQ ID NO:374)--;
line 13, after "sequence" insert --(SEQ ID NO:375)--;
line 18, after "sequence" insert --(SEQ ID NO:376)--;
line 21, after "sequence" insert --(SEQ ID NO:375)--;
line 26, after "sequence" insert --(SEQ ID NO:376)--.

Page 76, line 1, after "sequence" insert --(SEQ ID NO:377)--;
line 3, after "sequence" insert --(SEQ ID NO:378)--;
line 7, after "sequence" insert --(SEQ ID NO:379)--;
line 10, after "sequence" insert --(SEQ ID NO:380)--;
line 14, after "sequence" insert --(SEQ ID NO:59)--;
line 16, after "purinoceptor" insert --(SEQ ID NO:39)--.

In the Claims:

Please cancel claims 1-18 without prejudice and add the following new claims 19-26.

19. (new) A protein comprising an amino acid sequence represented by SEQ ID NO:54 or a salt thereof..

20. (new) A DNA which comprises a DNA having a nucleotide sequence coding for the protein as claimed in claim 19.

21. (new) The DNA as claimed in claim 20 which comprises a nucleotide sequence represented by SEQ ID NO:55.

22. (new) A vector which comprises the DNA as claimed in claim 21.

23. (new) A transformant which is transformed by the vector as claimed in claim 22.

24. (new) A method for producing a protein comprising an amino acid sequence represented by SEQ ID NO:54 or a salt thereof, which comprises cultivating the transformant as claimed in claim 23.

25. (new) A method for determining a ligand to the protein or a salt thereof as claimed in claim 19, which comprises contacting the protein or a salt thereof as claimed in claim 19, with a compound to be tested and measuring a receptor-mediated cell stimulating activity.

26. (new) A screening method for a novel compound or a salt thereof capable of inhibiting the binding of the protein or a salt thereof as claimed in claim 19 with a ligand, which comprises carrying out the comparison between:

- (i) at least one case where said ligand is contacted with the protein or a salt thereof as claimed in claim 19, and
- (ii) at least one case where said ligand together with a compound to be tested with the protein or a salt thereof as claimed in claim 19,

and measuring a receptor-mediated cell stimulating activity.

REMARKS

Please substitute the originally filed pages 238-270 with the substitute sheets (numbered **238-404**) attached hereto and renumber claims and abstract accordingly.

Applicants have submitted substitute pages 238-404 to include the Sequence Listing as part of this Application.

Applications have amended the Application to include the sequence identification number in the specification where reference is made to the sequence by use of the assigned identifier as required by 37 CFR §1.812(d). No new matter has been added by virtue of the amendment made to the specification.

Applicants enclose herewith a "Statement to Support Filing and Submission in Accordance with 37 C.F.R. §§1.821-1.825.

Claims 1-18 have been canceled without prejudice and new claims 19-26 have been added. No new matter has been added by virtue of these amendments. Basis for the new claims can be found in the specification and the claims of the original application.

For example, new claim 19 is supported by SEQ ID NO: 54, Example 20 (pages 232-234), and the disclosures on page 188, lines 29-31 of the specification, and the like.

New claims 20-21 are supported by SEQ ID NO: 55, Example 20 (pages 232-234), and the disclosures on page 188, lines 32-34 of the specification, and the like.

New claim 22 is supported by the disclosures on page 44, lines 1-2, and from page 134, line 22 to page 135, line 21 of the specification, Example 20 (pages 232-234), and the like.

New claim 23 is supported by the disclosures on page 44, lines 3-4, and from page 135, line 22 to page 136, line 25 of the specification, Example 20 (pages 232-234), and the like.

New claim 24 is supported by the disclosures on page 44, lines 5-9, and from page 136, line 26 to page 139, line 23 of the specification, Example 20 (pages 232-234), and the like.

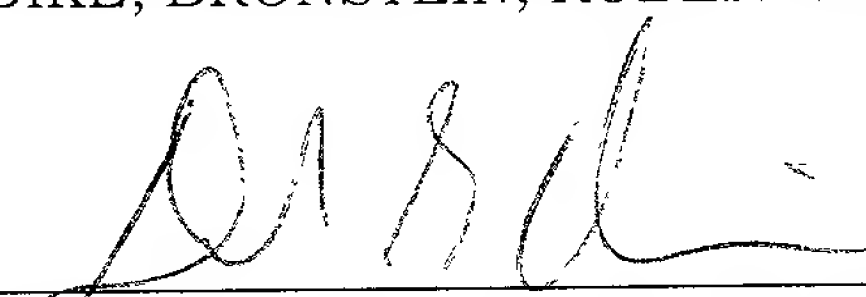
New claim 25 is supported by the disclosures on page 44, lines 10-20, and from page 140, line 29 to page 150, line 27 of the specification, and the like.

S. Hinuma, et al.
U.S.S.N.: Not Yet Assigned
Page 9

New claim 26 is supported by the disclosures on page 44, line 21 to page 45, line 1 and from page 155, line 23 to page 163, line 21 of the specification, and the like.

Respectfully submitted,

DIKE, BRONSTEIN, ROBERTS & CUSHMAN



Date: December 14, 1999

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130118

- 1 -

DESCRIPTION

G PROTEIN COUPLED RECEPTOR PROTEIN,
PRODUCTION, AND USE THEREOFFIELD OF THE INVENTION

5 The present invention relates to novel DNAs which are
useful as DNA primers for a polymerase chain reaction (PCR);
methods for amplifying DNAs each coding for a G protein
coupled receptor protein via PCR techniques using said DNA;
screening methods for DNAs each encoding a G protein coupled
10 receptor protein via PCR techniques using said DNA;
G protein coupled receptor protein-encoding DNAs obtained by
said screening method; G protein coupled receptor proteins
which are encoded by the DNA obtained via said screening
method, peptide fragments or segments thereof, and modified
15 peptide derivatives thereof; etc.

 The present invention also relates to novel G protein
coupled receptor proteins; novel G protein coupled receptor
protein-encoding DNAs; processes for producing said G protein
coupled receptor protein; use of said receptor protein and
20 said protein-encoding DNA; etc.

 The present invention also relates to novel human
amygdaloid nucleus-derived G protein coupled receptor proteins;
novel DNAs each coding for said G protein coupled receptor
protein; processes for producing said G protein coupled
25 receptor protein; use of said receptor protein and
said protein-encoding DNA; etc.

 The present invention also relates to novel mouse
pancreatic β cell line MIN6-derived G protein coupled receptor
proteins; novel DNAs each coding for said G protein coupled
30 receptor protein; processes for producing said G protein
coupled receptor protein; use of said receptor protein and

said protein-encoding DNA; etc. Further, the present invention relates to novel human-derived G protein coupled receptor proteins (human prinoceptors); novel DNAs each coding for said G protein coupled receptor protein; processes for producing
5 said G protein coupled receptor protein; use of said receptor protein and said protein-encoding DNA; etc.

BACKGROUND OF THE INVENTION

A variety of hormones, neurotransmitters and the like control, regulate or adjust the functions of living bodies via
10 specific receptors located in cell membranes. Many of these receptors mediate the transmission of intracellular signals via activation of guanine nucleotide-binding proteins (hereinafter, sometimes referred to as G proteins) with which the receptor is coupled and possess the common (homologous) structure, i.e.
15 seven transmembranes (membrane-spanning regions (domains)). Therefore, such receptors are generically referred to as G protein coupled receptors or seven transmembrane (membrane-spanning) receptors.

G protein coupled receptor proteins have a very
20 important role as targets for molecules such as hormones, neurotransmitters and physiologically active substances, which molecules control, regulate or adjust the functions of living bodies. Each molecule has its own receptor protein which is specific thereto, whereby the specificities of individual
25 physiologically active substances, including specific target cells and organs, specific pharmacological actions, specific action strength, action time, etc., are decided. Accordingly, it has been believed that, if G protein coupled receptor genes or cDNA can be cloned, those will be helpful not only for the
30 clarification of structure, function, physiological action, etc. of the G protein coupled receptor but also for the development of pharmaceuticals by investigating the substances which act on the receptor. Until now, only several G protein coupled receptor genes or cDNAs have been cloned but it is
35 believed that there are many unknown G protein coupled receptor genes which have not been recognized yet.

The characteristic feature of the G protein coupled receptor proteins which have been known up to now is that seven clusters of hydrophobic amino acid residues are located in the primary structure and pass through (span) the cell membrane at each region thereof. It has been known that such a structure is common among all of the known G protein coupled receptor proteins and further that the amino acid sequences corresponding to the area where the protein passes through the membrane (membrane-spanning region or transmembrane region) and the amino acid sequences near the membrane-spanning region are often highly conserved among the receptors.

When an unknown protein has such a structure, it is strongly suggested that said protein is within a category of the G protein coupled receptor proteins. In addition, some amino acid residue alignments are common (homologous) and, by taking it as a characteristic feature, it is further strongly suggested that said protein is a G protein coupled receptor protein.

Libert, F, et al. (Science, 244:569-571; 1989) reported a method for cloning novel receptor genes by means of a polymerase chain reaction (hereinafter, sometimes referred to as PCR or a PCR technique) for a synthetic DNA primer which was synthesized based upon the information of common amino acid sequences obtained from a comparison among known G protein coupled receptor proteins. Libert, F. et al. used a pair of synthetic DNA primers corresponding to the portions of the third and the sixth membrane-spanning regions. However, in general, the design of primers used for the PCR regulates the molecular species of DNAs which are to be amplified.

In addition, when a similarity (homology) in the amino acid sequence level is used as a basis, the use of different codons affects on the binding (hybridization) of the primer thereby resulting in a decrease in the amplifying efficiency.

Accordingly, although various novel receptor protein DNAs have been obtained using said DNA primers, it is not possible to succeed in amplifying DNAs for all receptor proteins in the prior art.

Further, the amino acid sequence which is common to from the first to the seventh membrane-spanning regions among 74 G protein coupled receptor proteins was reported by William C. Probst, et al. (DNA and Cell Biology, Vol. 11, No. 1, 1992, pp. 1-20). In this report, however, there is no suggestion for a method in which DNA coding for a novel G protein coupled receptor protein is screened by means of PCR using DNA primers which are complementary to the DNA coding for those amino acid sequences.

It would be desirable to develop DNA primers for PCR techniques which allow selective and efficient screenings of DNAs coding for the areas (regions) more nearer the full length of novel G protein coupled receptor proteins by utilizing the common (homologous) sequence(s) of the G protein coupled receptor protein or the DNA coding therefor.

It would also be desirable to develop synthetic DNA primers corresponding to the portions of the third and the sixth membrane-spanning regions, said primer being useful in screening for DNA coding for G protein coupled receptor proteins in more selective and efficient manner as compared with a series of the synthetic DNA primers corresponding to the sequences of the third, to the sixth membrane-spanning regions as reported by Libert, F. et al.

G protein coupled receptor proteins are important for investigating substances which control the function of living organisms and proceeding developments thereof as pharmaceuticals. Finding and development of candidate compounds for new pharmaceuticals can be efficiently proceeded by using G protein coupled receptor proteins and by conducting receptor binding experiments and evaluating experiments on agonists/antagonists using intracellular information transmittance systems as indexes. Especially when the presence of a novel G protein coupled receptor protein can be clarified, the presence of a substance having a specific action thereon can be suggested.

If a novel DNA which codes for a novel G protein coupled receptor protein can be efficiently screened and

isolated, it will now be possible to proceed with the isolation of DNA having an entire coding region, the construction of an expression system therefor and the screening of an acting ligand.

5 A hypothalamo-hypophysial system is one of the passages for controlling, regulating or adjusting the functions of organisms relying upon interactions of hormones and neurotransmitters with G protein coupled receptors. In the hypothalamo-hypophysial system, the secretion of pituitary hormones from the pituitary body (hypophysis) is regulated by hypothalamic hormones (hypophysiotropic releasing factors), and the functions of target cells and organs are controlled by pituitary hormones released into the blood. Functions which are important for the living body are regulated through this system, such as maintenance of homeostasis and control of development and growth of a genital system and an individual organism. Representative examples of the hypothalamic hormones include TRH, LH-RH, CRF, GRF, somatostatin, galanin, etc. Representative examples of the pituitary hormones include TSH, ACTH, FSH, LH, prolactin, growth hormone, oxytocin, vasopressin, etc. In particular, the secretion of pituitary hormones is regulated according to a positive feedback mechanism or a negative feedback mechanism relied on the hypothalamic hormones and peripheral hormones secreted from the target endocrine glands. A variety of receptor proteins present in the pituitary gland play a major role for regulating the hypothalamo-hypophysial system.

 It has been widely known that these hormones, factors and receptors are widely distributed in the brain instead of existing only locally in the hypothalamo-hypophysial system. This fact suggests that the substances which are called "hypothalamic hormones" are working as neurotransmitters or neuroregulators in the central nervous system. It is further considered that these substances are similarly distributed even in the peripheral tissues to play the role of important functions. The pancreas plays an important role of carrying out the carbohydrate metabolism by secreting not only

a digestive fluid but also glucagon and insulin. Insulin is secreted from the β cells and its secretion is promoted chiefly by glucose. It has, however, been known that a variety of receptors exist in the β cells, and the secretion of insulin is controlled by various factors such as peptide hormones (galanin, somatostatin, gastric inhibitory polypeptide, glucagon, amylin, etc.), sugars (mannose, etc.), amino acids, and neurotransmitters in addition to glucose.

It has thus been known that in the pituitary gland and in the pancreas are present receptor proteins for many hormones and neurotransmitters, said receptor proteins playing important roles for regulating the functions. As for the galanin and amylin, however, there has not yet been reported any discovery concerning the structure of their receptor protein cDNAs. It is not known whether there exist any unknown receptor proteins or receptor protein subtypes.

For substances regulating the functions of the pituitary gland and pancreas, there exist receptor proteins specific to said substance on the surfaces of various functional cells of the pituitary gland and pancreas. The pituitary gland and the pancreas are associations of a plurality of functional cells, and the actions of the individual substances are defined by the distributions of their target receptor proteins among the functional cells.

Accordingly, a substance, in many cases, exhibits an extensive variety of actions. To comprehend such complex systems, it is necessary to clarify the relations between the acting substances and the specific receptor proteins. It is further necessary to efficiently screen for receptor protein agonists and antagonists capable of regulating the pituitary gland and pancreas, to clarify the structures of genes of receptor proteins from the standpoint of investigating and developing pharmaceuticals, and further to express them in a suitable expression system.

By utilizing the fact that a G protein coupled receptor protein exhibits homology in part of the structure thereof at the amino acid sequence level, an experiment of

looking at DNAs coding for novel receptor proteins relying upon a polymerase chain reaction (hereinafter simply referred to as "PCR") has recently been made.

5 In the central nervous system, many receptor proteins such as dopamine receptor protein, LH-RH receptor protein, neurotensin receptor protein, opioid receptor protein, CRF receptor protein, CRF receptor protein, somatostatin receptor protein, galanin receptor protein, TRH receptor protein, etc. are G protein coupled receptor proteins, and it has been
10 clarified that ligands to these receptors exert a variety of effects in the central nervous system.

In the immune system, an α + or a β -chemokine receptor protein, an MIP1 α receptor protein, an IL-8 receptor protein, a C5a receptor protein, etc. have been known as such
15 G protein coupled receptor proteins, and are working as receptor proteins responsive to immunoregulating substances to play important roles for regulating the functions of the living body. There is, for example, an IL-6 receptor protein that acts both in the above-mentioned central nervous system and in
20 the immune system. IL-6 is both a β -cell differentiating factor and a biologically active factor related to the proliferation and differentiation of nerve cells.

It has been widely known that these hormones, factors and receptor proteins are usually widely distributed
25 up to the peripheral tissues instead of existing only locally in the central nervous system and in the immune system and are producing important functions, respectively. Agonists and antagonists for these receptor proteins are now being developed as various useful pharmaceuticals.

30 For substances regulating the functions of the central nervous system and the immune system, there exist receptor proteins specific to said substance on the surfaces of various functional cells of the central nervous system and the immune system. The central nervous system and the immune
35 system are associations of a plurality of functional cells, and the actions of the individual substances are defined by the distributions of their target receptor proteins among the

functional cells. Accordingly, a substance, in many cases, exhibits an extensive variety of actions. Moreover, there is an example wherein many factors play a part in a physiological phenomenon. To comprehend such complex systems, it is necessary to clarify relations between the acting substances and the specific receptor proteins.

As discussed herein above, the G protein coupled receptor protein is present on the cell surface of living body cells and organs and has a very important role as a target for molecules such as hormones, neurotransmitters and physiologically active substances, which molecules control, regulate or adjust the functions of living body cells and organs.

SUMMARY OF THE INVENTION

One object of the present invention is to provide novel DNAs which are useful as DNA primers for a polymerase chain reaction; methods for amplifying a DNA coding for a G protein coupled receptor protein using said DNA; screening methods for the DNA coding for a G protein coupled receptor protein using said DNA; DNAs obtained by said screening method; and G protein coupled receptor proteins encoded by the DNA obtained by said screening method, peptide fragments or segments thereof, modified peptide derivatives thereof or salts thereof.

Another object of the present invention is to provide processes for producing said receptor protein; transformants capable of expressing said receptor protein; cell membrane fractions obtained from said transformant; methods for determining a ligand to the receptor protein; screening methods for a compound or a salt thereof capable of inhibiting the binding of the ligand with the receptor protein; kits for said screening method, pharmaceutical compositions comprising an effective amount of the inhibitory compound; antibodies against said receptor protein; immunoassays using said receptor protein or said antibody and use of said receptor protein and encoding DNA.

Yet another object of the present invention is to provide novel G protein coupled receptor proteins which are expressed in pituitary glands or pancreatic β cells; DNAs comprising a DNA coding for said G protein coupled receptor protein; processes for producing said receptor protein; transformants capable of expressing said receptor protein; cell membrane fractions obtained from said transformant; methods for determining a ligand to the receptor protein; screening methods for a compound or a salt thereof capable of inhibiting the binding of the ligand with the receptor protein; kits for said screening method, pharmaceutical compositions comprising the inhibitory compound; antibodies against said receptor protein; immunoassays using said receptor protein or said antibody and use of said receptor protein and encoding DNA.

Still another object of the present invention is to provide novel human amygdaloid nucleus-derived G protein coupled receptor proteins; DNAs comprising a DNA coding for said G protein coupled receptor protein; processes for producing said receptor protein; transformants capable of expressing said receptor protein; cell membrane fractions obtained from said transformant; methods for determining a ligand to the receptor protein; screening methods for a compound or a salt thereof capable of inhibiting the binding of the ligand with the receptor protein; kits for said screening method, pharmaceutical compositions comprising the inhibitory compound; antibodies against said receptor protein; immunoassays using said receptor protein or said antibody and use of said receptor protein and encoding DNA.

Yet another object of the present invention is to provide novel mouse pancreatic β cell line MIN6-derived G protein coupled receptor proteins; DNAs comprising a DNA coding for said G protein coupled receptor protein; processes for producing said receptor protein; transformants capable of expressing said receptor protein; cell membrane fractions obtained from said transformant; methods for determining a ligand to the receptor protein; screening methods for a

compound or a salt thereof capable of inhibiting the binding of the ligand with the receptor protein; kits for said screening method, pharmaceutical compositions comprising the inhibitory compound; antibodies against said receptor protein; immunoassays using said receptor protein or said antibody and use of said receptor protein and encoding DNA.

The present inventors have succeeded in synthesizing novel DNA primers based upon the similarity (homology) with the base sequences coding for the first membrane-spanning region or the sixth membrane-spanning region each of known G protein coupled receptor proteins. It is to be particularly noted that there has been no report of a DNA primer pair which has been synthesized paying attention to the similarity with the base sequence coding for the first and the sixth membrane-spanning region of the known G protein coupled receptor protein.

Next the present inventors have succeeded in synthesizing other novel DNA primers based upon the similarity (homology) with the base sequences coding for the third or the sixth membrane-spanning region each of known G protein coupled receptor proteins. They have also unexpectedly succeeded in efficiently amplifying DNAs (DNA fragments) coding for G protein coupled receptor proteins by means of PCR using those DNA primers.

They have further succeeded in synthesizing novel DNA primers based upon the similarity (homology) with the base sequences coding for the second or the seventh membrane-spanning region each of known G protein coupled receptor proteins; upon the similarity (homology) with the base sequences coding for first or the third membrane-spanning region each of known G protein coupled receptor proteins; and upon the similarity (homology) with the base sequences coding for the second or the sixth membrane-spanning region each of known G protein coupled receptor proteins. They have furthermore and unexpectedly succeeded in efficiently amplifying DNAs (DNA fragments) coding for G protein coupled receptor proteins by conducting PCR using those DNA primers.

Moreover, the present inventors have succeeded in

efficiently cloning full-length DNA coding for said G protein coupled receptor protein via using amplified DNAs (DNA fragments) coding for said G protein coupled receptor protein. Thus, they have found that novel DNA coding for novel G protein coupled receptor proteins can be isolated, characterized or prepared via conducting amplifications and analyses of various DNA using said DNA primers.

To be more specific, the present inventors have selected amino acid sequences which are each common to the portion corresponding to or near the first and the sixth membrane-spanning region of the known individual G protein coupled receptor proteins and have designed the DNA primer (SEQ ID NO: 1) coding for the amino acid sequence common (homologous) to the first membrane-spanning region and the DNA primer (SEQ ID NO: 2) which is complementary to the nucleotide sequence coding for the amino acid sequence common (homologous) to the area near the sixth membrane-spanning region. Those DNA primers have a different nucleotide sequence as compared with reported DNA primers (e.g. a set of synthetic DNA primers corresponding to the third and the sixth membrane-spanning regions (SEQ ID NO: 60 and SEQ ID NO: 61) as reported by Libert, F. et al.) and such instant primers are novel and unique.

Especially for an object of conducting an efficient elongation reaction in the PCR, the 3'-terminal region of the instant primers contains the nucleotide sequence which is common (homologous) among many receptor proteins. Even in other areas, the similarity (homology) at the nucleotide sequence level (base sequence level) is utilized for setting the mixed base (nucleotide) parts wherein their nucleotide sequences (base sequences) are matched for as many nucleotides (bases) as possible among many DNA for the receptor proteins. Then the present inventors have amplified cDNA derived from human brain amygdala, human pituitary gland and rat brain, found the amplified products as shown in Figure 17 and, from those products, obtained the G protein coupled receptor protein cDNAs having the sequence as shown in

Figure 18, Figure 19, Figure 20, Figure 21, Figure 22,
Figure 23, Figure 27, Figure 29, Figure 34, Figure 37,
Figure 40, Figure 43 or Figure 46. Among them, the G protein
coupled receptor protein cDNAs having the sequence as shown in
5 Figure 22, Figure 23, Figure 27, Figure 29, Figure 34,
Figure 37, Figure 40, Figure 43 or Figure 46 are novel.

Further, the present inventors have selected the
amino acid sequences common (homologous) to the third and the
sixth membrane-spanning region each of the known G protein
10 coupled receptor proteins and designed the DNA primers coding
for the amino acid sequence common (homologous) to the third
membrane-spanning region (SEQ ID NO: 3; SEQ ID NO: 5, SEQ ID
NO: 6 and SEQ ID NO: 7) and the DNA primers which are
complementary to the nucleotide sequence coding for the amino
15 acid sequence common (homologous) to the portion near the sixth
membrane-spanning region (SEQ ID NO: 4, SEQ ID NO: 8 and SEQ ID
NO: 9). Again, those DNA primers have different base sequences
from those of the DNA primers previously reported (e.g., a set
of synthetic DNA primers corresponding to the sequence of the
20 third and the sixth membrane-spanning regions (SEQ ID NO: 60
and SEQ ID NO: 61) as reported by Libert, F. et al.) and such
instant primers are novel and unique. The present inventors
amplified cDNA derived from the smooth muscles of gastric
pylorus of rabbits using said DNA primer and obtained G protein
25 coupled receptor protein cDNA having the sequence of Figure 49
or Figure 52. Those cDNAs are novel.

Still further, the present inventors have selected
the amino acid sequences common (homologous) to the second and
the seventh membrane-spanning region each of the known G
30 protein coupled receptor proteins and designed the DNA primer
coding for the amino acid sequence common (homologous) to the
second membrane-spanning region (SEQ ID NO: 10) and the DNA
primer which is complementary to the base sequence coding for
the amino acid sequence common (homologous) to the portions
35 near the seventh membrane-spanning region (SEQ ID NO: 11).
Those DNA primers have different base sequences from those of
DNA primers previously reported (e.g., a set of synthetic DNA

primers corresponding to the part of the third and the sixth membrane-spanning regions (SEQ ID NO: 60 and SEQ ID NO: 61) as reported by Libert, F. et al) and such instant primers are novel and unique. The present inventors amplified cDNA derived from the smooth muscles of gastric pylorus of rabbits using said DNA primer and obtained G protein coupled receptor protein cDNAs having each the sequence of Figure 55, Figure 56, Figure 72, or Figure 73. Those cDNAs are novel.

Furthermore, the present inventors have selected the amino acid sequences common (homologous) to the first and the third membrane-spanning region each of the known G protein coupled receptor proteins and designed the DNA primer coding for the amino acid sequence common (homologous) to the first membrane-spanning region (SEQ ID NO: 12) and the DNA primer which is complementary to the base sequence coding for the amino acid sequence common (homologous) to the portions near the third membrane-spanning region (SEQ ID NO: 13).

Still further, the present inventors have selected the amino acid sequences common (homologous) to the third and the sixth membrane-spanning region each of the known G protein coupled receptor proteins and designed the DNA primers coding for the amino acid sequence common (homologous) to the third membrane-spanning region (SEQ ID NO: 10 and SEQ ID NO: 18) and the DNA primers which are complementary to the base sequence coding

for the amino acid sequence common (homologous) to the parts near the sixth membrane-spanning region (SEQ ID NO: 15 and SEQ ID NO: 19). Further, the present inventors have selected the amino acid sequences common (homologous) to the second and the sixth membrane-spanning region each of the known G protein coupled receptor proteins and designed the DNA primer coding for the amino acid sequence common (homologous) to the second membrane-spanning region (SEQ ID NO: 16) and the DNA primer which is complementary to the base sequence coding for the amino acid sequence common (homologous) to the parts near the sixth membrane-spanning region (SEQ ID NO: 17). Those DNA primers have different base sequences from those of DNA primers previously reported (e.g., a set of synthetic DNA primers

corresponding to the part of the third and the sixth membrane-spanning regions (SEQ ID NO: 60 and SEQ ID NO: 61) as reported by Libert, F. et al) and such instant primers are novel and unique.

5 Still another object of the present invention is to provide a G protein coupled receptor protein expressed in the pituitary gland and pancreatic β cells, a DNA comprising a DNA coding for said protein, a process for producing said protein, and use of said protein and DNA.

10 In order to achieve the above-mentioned aims, the present inventors have made extensive investigations. As a result, the present inventors have succeeded in amplifying cDNA derived from the human pituitary gland and the mouse pancreatic β -cell strain, MIN 6, with a synthetic DNA primer
15 for efficiently isolating G protein coupled receptor protein-encoding DNA, and have forwarded the analysis. Thus, the present inventors have succeeded in isolating novel human and mouse-derived G protein coupled receptor protein-encoding cDNAs, in determining the partial structure thereof, and have
20 considered that these cDNA sequences are preserved very well in the human and in the mouse, and are coding for novel receptor proteins for the same ligand. Based upon the above knowledge, the present inventors have discovered that these DNAs make it possible to obtain a cDNA having a full length
25 open reading frame (ORF) of the receptor protein, hence, to produce the receptor protein. The inventors have further discovered that the above-mentioned receptor protein obtained when the G protein coupled receptor protein-encoding cDNA is expressed by a suitable means permits screening for a ligand to
30 the receptor protein from the living body or from natural or non-natural compounds under guidance of data obtainable in receptor coupling tests or measurements of intracellular second messengers, etc. and further allows screening for a compound that inhibits the binding of the ligand and the
35 receptor protein.

 In one embodiment, the present inventors have carried out PCR amplification of novel human pituitary gland-

derived cDNA fragments as shown in Figures 22 and 23, and have subcloned them to obtain a plasmid vector (p19P2).

From analysis of the partial sequence, it has been clarified that the cDNA has been encoded a novel receptor protein.

5 The synthetic DNA primers used for amplifying the cDNA are corresponding to seven hydrophobic clusters that exist in the known G protein coupled receptor proteins in common, i.e., corresponding to the first and sixth membrane-spanning regions among the membrane-spanning domains. The nucleotide sequence
10 (SEQ ID NO: 29) has been determined from the primer region at the 5' side (first membrane-spanning domain side) and has been translated into an amino acid sequence (SEQ ID NO: 24)

[Figure 22]. As a result, the second and third membrane-spanning domains have been confirmed on the hydrophobicity plotting [Figure 58]. Similarly, the nucleotide sequence
15 (SEQ ID NO: 30) has been determined from the primer region at the 3' side (sixth membrane-spanning domain side) and has been translated into an amino acid sequence (SEQ ID NO: 25)

[Figure 23]. As a result, the presence of the sixth and fifth
20 membrane-spanning domains has been confirmed on the hydrophobicity plots [Figure 59]. The size of the amplified cDNA is about 700 bp which is nearly comparable with the number of bases between the first membrane-spanning domain and the sixth membrane-spanning domain of the known G protein coupled
25 receptor protein.

G protein coupled receptor proteins exert common property to some extent at an amino acid sequence level, and are forming one protein family. Therefore, data base retrieval has been carried out based upon the amino acid sequence of the
30 subject novel receptor protein (protein encoded by cDNA included in p19P2). As a result, a high homology has been exhibited as compared with the known G protein coupled receptor protein (rat neuropeptide Y receptor protein encoded by S12863) that is shown in Figure 60. This fact tells that
35 the novel receptor protein of the present invention belongs to the G protein coupled receptor protein family. Moreover, the data base has been retrieved using, as a template, the amino

acid sequence encoded by the DNA of the invention. It exhibits high homology to the amino acid sequences of the known G protein coupled receptor proteins, mouse-derived ligand unknown RP-23 (B40470), human-derived ligand unknown K-opioid receptor protein (P30098) and human-derived NK-2 receptor protein (JQ1059). However, none of them are in perfect agreement, from which it is learned that a novel receptor protein had been encoded. The aforementioned abbreviations in parentheses are reference numbers that are assigned when they are registered as data to NBRF-PIR/Swiss-PROT and are, usually, each called "Accession Number".

Next, by using the novel G protein coupled receptor protein-encoding cDNA fragment (p19P2) of the present invention, a cDNA having a full-length open reading frame of the receptor protein of the present invention has been obtained from human pituitary gland cDNA libraries. The nucleotide sequence analysis of a plasmid (phGR3) carrying the cDNA having a full length open reading frame of the receptor protein shows that the nucleotide sequence of a coding region of this receptor protein is represented by SEQ ID NO: 31, and the amino acid sequence deduced therefrom is represented by SEQ ID NO: 26 [Figure 34]. Based upon the amino acid sequence, hydrophobicity plotting has been carried out. The results are shown in Figure 36. From the hydrophobicity plotting, it has been clarified that the receptor protein of the present invention possessed seven hydrophobic domains. That is, it has been confirmed that the receptor protein encoded by the cDNA obtained according to the present invention is a seven transmembrane (membrane-spanning) G protein coupled receptor protein. An expression of mRNA for receptor genes encoded by the cDNA of the present invention has been checked by northern blotting techniques at a mRNA level, and it has been confirmed that the receptor gene has been expressed in the human pituitary gland [Figure 35].

The present inventors have further succeeded in PCR amplification of a mouse pancreatic β cell strain, MIN6 derived cDNA fragment, and cloning of pG3-2 and pG1-10.

Then, based on the nucleotide sequence of cDNA included in these two plasmid vectors, the nucleotide sequence shown in Figure 27 has been derived. It was learned from the nucleotide sequence that the cDNA encodes a novel receptor protein.

5 Upon translating the nucleotide sequence into an amino acid sequence, the presence of the third, fourth, fifth and sixth membrane-spanning domains has been confirmed on the hydrophobicity plots [Figure 28]. The size of the amplified cDNA is about 400 bp which is nearly comparable with the number
10 of bases between the third membrane-spanning domain and the sixth membrane-spanning domain of the known G protein coupled receptor protein. The amino acid sequence has been compared with amino acid sequences [Figures 22 and 23] encoded by the G protein coupled receptor protein cDNA included in p19P2 cloned from the human pituitary gland. As a result, homology is more
15 than 95% [Figure 61]. From this fact, it was estimated that the protein encoded by the cDNA included in pG3-2 is a mouse type G protein coupled receptor protein relative to the human-derived one encoded by the cDNA included in p19P2.

20 The present inventors have further amplified a mouse pancreatic β -cell strain, MIN6-derived cDNA fragment by the PCR followed by subcloning into a plasmid vector to obtain a clone (p5S38) having a nucleotide sequence as shown in Figure 62. From the nucleotide sequence (SEQ ID NO: 33), it
25 has been clarified that the cDNA encodes a novel receptor protein. Upon translating the nucleotide sequence into an amino acid sequence (SEQ ID NO: 28), the presence of the third, fourth, fifth and sixth membrane-spanning domains has been confirmed on the hydrophobicity plots [Figure 64]. The size of
30 the amplified DNA is about 400 bp that is nearly comparable with the known G protein coupled receptor protein. The amino acid sequence has been compared with amino acid sequences [Figures 22 and 23] encoded by the G protein coupled receptor protein cDNA included in p19P2 cloned from the human pituitary
35 gland and with amino acid sequences of proteins encoded by pG3-2 and pG1-10 derived from the mouse pancreatic β -cell strain. As a result, homology is more than 95% to them

[Figure 63]. This fact suggests that the protein encoded by the human-derived pituitary gland-derived p19P2, the proteins encoded by the mouse pancreatic β -cell strain-derived pG3-2 and pG1-10, and the protein encoded by the mouse pancreatic β -cell strain-derived p5S38, pertain to a receptor family that recognizes the same ligand.

Another object of the present invention is to provide a novel human amygdaloid nucleus-derived protein coupled receptor protein, a DNA containing a DNA coding for said G protein coupled receptor protein, a process for producing said G protein coupled receptor protein, and use of said protein and DNA.

The present inventors have synthesized DNA primers for efficiently isolating a DNA coding for G protein coupled receptor proteins, amplified an amygdaloid nucleus-derived cDNA with the above primer, and have analyzed it.

As a result, the present inventors have succeeded in isolating, from the human amygdaloid nucleus, a cDNA coding for a novel G protein coupled receptor protein and have determined its partial structure. The nucleotide sequence of the isolated cDNA is preserved very well as compared with that of the mouse glucocorticoid-induced receptor (hereinafter sometimes referred to as "GIR") and is considered to be encoding a receptor protein to the same ligand (Molecular Endocrinology 5:1331-1338, 1991). It is reputed that, in the mouse, the GIR is a receptor which is induced by glucocorticoid and expressed in T-cells and is working as a receptor to immunoregulating factors in the immune system on the T-cells. The present inventors have succeeded in the isolation of this human type GIR from the human amygdaloid nucleus. Accordingly, it is suggested that the isolated GIR is expressed even in the human central nervous system to carry out some function. From these facts, it is considered that the receptor protein is strongly expressed in the human brain and in the immune system and is also functioning therein. These characterized DNAs allow one to obtain a cDNA having a full length open reading frame of the receptor and production of the receptor

proteins. The receptor proteins expressed by a suitable means, furthermore, permit screening for a ligand to the receptor proteins from the living body or from natural and non-natural compounds depending on indications obtainable in receptor protein-binding experiments, measurements of intracellular second messengers, etc. It further allows one to screen for compounds capable of inhibiting the binding between the ligand and the receptor protein.

To be more specific, the present inventors have amplified, as a novel human amygdaloid nucleus-derived cDNA, one species, as shown in Figures 29 and 30, by PCR, cloned it, and clarified from the analysis of a partial sequence thereof that a novel receptor protein is encoded. The synthetic DNA primers used for amplifying the cDNA are corresponding to seven hydrophobic clusters that exist in the G protein coupled receptor proteins in common, i.e., corresponding to the first and sixth membrane-spanning regions among the membrane-spanning domains. The nucleotide sequence has been determined from the primer region at the 5' side (first membrane-spanning domain side) and has been translated into an amino acid sequence. As a result, the second and third membrane-spanning domains have been confirmed on the hydrophobicity plotting [Figure 31]. Similarly, the nucleotide sequence has been determined from the primer region at the 3' side (sixth membrane-spanning domain side) and has been translated into an amino acid sequence. As a result, the presence of the fifth and fourth membrane-spanning domains has been confirmed on the hydrophobicity plots [Figure 32]. The size of the amplified cDNA is about 700 bp which is nearly comparable with the number of bases of the known G protein coupled receptor protein.

The inventors have further retrieved the data base based on, as a template, the nucleotide sequence of the isolated DNA and observed high homology to the DNA that codes for mouse-derived glucocorticoid-induced receptor protein which is a widely known G protein coupled receptor protein [Figure 33]. This result strongly suggests that the DNA of the present invention is encoding a human-type receptor protein of GIR.

Yet another object of the present invention is to provide a novel mouse pancreatic β -cell strain, MIN6-derived protein coupled receptor protein, a DNA containing a DNA coding for said G protein coupled receptor protein, a process for producing said G protein coupled receptor protein, and use of said protein and DNA. The present inventors have synthesized DNA primers for efficiently isolating a DNA coding for G protein coupled receptor proteins, amplified a mouse pancreatic β -cell strain, MIN6-derived cDNA with the above primer, and have analyzed it.

As a result, the present inventors have succeeded in isolating a mouse-derived cDNA coding for a novel G protein coupled receptor protein and have determined its partial structure. The isolated cDNA is homologous to known G protein coupled receptors at the nucleotide sequence level and at the amino acid sequence level and is considered to be encoding a novel receptor protein which is expressed in the mouse pancreas and is also functioning therein. These characterized DNAs allow one to obtain a cDNA having a full length open reading frame of the receptor and production of the receptor proteins. Human-derived cDNAs may be cloned by using, as a probe, said mouse-derived cDNA. The receptor proteins expressed by a suitable means, furthermore, permit screening for a ligand to the receptor protein from the living body or from natural and non-natural compounds relying on indications obtainable in receptor protein-binding experiments, measurements of intracellular second messengers, etc. It further allows one to screen for compounds capable of inhibiting the binding of the ligand with the receptor protein.

To be more specific, the present inventors have amplified, as a novel mouse pancreatic β -cell strain, MIN6-derived cDNA, p3H2-17, as shown in Figures 37, by PCR, cloned it, and clarified from the analysis of a partial sequence thereof that a novel receptor protein is encoded. The nucleotide sequence has been translated into an amino acid sequence. As a result, the presence of the third, fourth, fifth and sixth membrane-spanning domains has been confirmed

on the hydrophobicity plots [Figure 38]. The size of the amplified cDNA is about 400 bp which is nearly comparable with that of the known G protein coupled receptor protein.

The inventors have retrieved the data base based on, as a template, the nucleotide sequence of the isolated DNA and observed 30% homology to chicken ATP receptor (P34996), 25% homology to human somatostatin receptor subtype 3 (A46226), 27% homology to human somatostatin receptor subtype 4 (JN0605), and 28% homology to bovine neuropeptide Y receptor (S28787), respectively (Figure 39), which are known G protein coupled receptor proteins. The aforementioned abbreviations in parentheses are reference numbers that are assigned when they are registered as data to NBRF-PIR/Swiss-PROT and are, usually, each called "Accession Number".

An expression of receptor genes encoded by the cDNA fragment included in p3H2-17 of the present invention has been checked by northern blotting techniques at a mRNA level, and it has been confirmed that the receptor gene has been intensely expressed in the mouse thymus and spleen. It has been also confirmed that the receptor gene has been expressed in the mouse brain and pancreas (Figure 65).

Next, by utilizing the information on the nucleotide sequence of the fragment included in p3H2-17, cDNA encoding a full-length open reading frame of the mouse pancreatic β -cell strain, MIN6-derived G protein coupled receptor protein of the present invention has been obtained from mouse thymic and splenic poly(A)⁺ RNA by 5'RACE (5' rapid amplification of cDNA ends) techniques (Frohman M.A. et al., Proc. Natl. Acad. Sci. USA, 85:8998-9002 (1988); Belyavsky A. et al., Nucleic Acids Res., 17:2919-2932 (1989); Edwards J.B.D.M. et al., Nucleic Acids Res., 19:5227-5232 (1991)) and 3'RACE (3' rapid amplification of cDNA ends) techniques (Frohman M.A. et al., Proc. Natl. Acad. Sci. USA, 85:8998-9002 (1988); Belyavsky A. et al., Nucleic Acids Res., 17:2919-2932 (1989)).

The plasmid (pMAH2-17) carrying cDNA encoding a full-length open reading frame of the receptor protein of the

present invention has been subjected to sequencing analysis. As a result, the nucleotide sequence of the region coding for the receptor protein is represented by SEQ ID NO: 41 and the amino acid sequence deduced therefrom is represented by SEQ ID NO: 39 (Figure 69). Based on the amino acid sequence, hydrophobicity plotting has been carried out. The results are shown in Figure 70.

It has been clarified from the hydrophobicity plotting that the mouse pancreatic β -cell strain, MIN6-derived receptor protein of the present invention has seven hydrophobic domains. Thus, it has been confirmed that the receptor protein encoded by the cDNA included in pMAH2-17 according to the present invention is a seven transmembrane G protein coupled receptor protein.

Data base retrieval has been carried out based on the full-length amino acid sequence encoded by the cDNA included in pMAH2-17, and it has been observed that the amino acid sequence has 44.0% homology to mouse P_{2U} purinoceptor (P35383) and 38.1% homology to chicken P_{2Y} purinoceptor (P34996), respectively (Figure 71), which are known G protein coupled receptor proteins. The aforementioned abbreviations in parentheses are reference numbers that are assigned when they are registered as data to NBRF-PIR/Swiss-PROT and are, usually, each called "Accession Number".

Since the receptor protein encoded by pMAH2-17 is highly homologous to prinoceptors, it is considered that there are strong possibility of a subtype within prinoceptor families. Therefore, the present inventors have carried out an electrophysiological analysis of the receptor gene in Xenopus oocytes and found significant inward currents elicited by Xenopus oocytes carrying the subject receptor gene in response to ATP stimulation (Figure 75). As a result, it has been determined that the receptor encoded by pMAH2-17 is one of the subtypes within prinoceptor families. It has been discussed and expected that there are a variety of subtypes among purinoceptors (Pharmac. Ther., Vol. 64, pp. 445-475 (1994)).

All data are supporting that the mouse pancreatic β -cell strain, MIN6-derived receptor protein of the present invention (e.g., SEQ ID NO: 38 and SEQ ID NO: 39, or proteins encoded by pMAH2-17) is a novel purinoceptor subtype which is clearly distinct from chicken P_{2y1} purinoceptor (FEBS LETTERS, Vol. 324(2), 219-225 (1993)); mouse P_{2y2} or P_{2u} purinoceptor (Proc. Natl. Acad. Sci. USA, Vol. 90, pp.5113-5117 (1993)); rat P_{2u} or P_{2y2} purinoceptor (Am. J. Respir. Cell Mol. Biol., Vol. 12, pp. 27-32 (1995)); human P_{2u} or P_{2y2} purinoceptor (Proc. Natl. Acad. Sci. USA, Vol. 91, pp.3275-3279 (1994)); and rat P_{2x} purinoceptor (Nature, Vol. 371.6, pp.516-519 (1994)).

It is also strongly suggested that agonists and/or antagonists related to the receptor encoded by pMAH2-17 would be useful in therapeutic or prophylactic treatment of diseases or syndromes in connection with purine ligand compounds. It is expected that the agonists of the receptor encoded by pMAH2-17 are useful as an immunomodulator or an antitumor agent, in addition they are useful in therapeutically or prophylactically treating hypertension, diabetes, cystic fibrosis, etc. It is still expected that the antagonists of the receptor encoded by pMAH2-17 are useful as hypotensive agents, analgesics, agents, for therapeutically or prophylactically treating incontinence of urine, etc.

Another object of the present invention is to provide a novel human-derived protein coupled receptor protein of prinoceptor type, a DNA containing a DNA coding for said G protein coupled receptor protein, a process for producing said G protein coupled receptor protein, and use of said protein and DNA. The present inventors have synthesized DNA primers for efficiently isolating a DNA coding for prinoceptor type G protein coupled receptor proteins on the basis of the nucleotide sequence of mouse purinoceptor, amplified a human-derived cDNA with the above primer, and have analyzed it.

As a result, the present inventors have succeeded in isolating a human-derived cDNA coding for a novel G protein coupled receptor protein and have determined its full-length structure [Figure 77]. The isolated cDNA is homologous to

mouse G protein coupled receptor (purinoceptor) at the nucleotide sequence level and at the amino acid sequence level (87% homology; Figure 79) and is considered to be encoding a novel purinoceptor protein. The receptor proteins expressed by a suitable means, furthermore, permit screening for a ligand to the receptor protein from the living body or from natural and non-natural compounds relying on indications obtainable in receptor protein-binding experiments, etc. It further allows one to screen for compounds capable of inhibiting the binding of the ligand with the receptor protein.

It is also strongly suggested that agonists and/or antagonists related to the human receptor encoded by phAH2-17 would be useful in therapeutic or prophylactic treatment of diseases or syndromes in connection with purine ligand compounds. It is expected that the agonists of the human receptor are useful as an immunomodulator or an antitumor agent, in addition they are useful in therapeutically or prophylactically treating hypertension, diabetes, cystic fibrosis, etc. It is still expected that the antagonists of the human receptor are useful as hypotensive agents, analgesics, agents for therapeutically or prophylactically treating incontinence of urine, etc.

Accordingly, one aspect of the present invention is

(1) DNAs comprising a nucleotide sequence represented by a SEQ ID NO selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 19;

(2) DNAs according to the above (1) comprising a nucleotide sequence represented by a SEQ ID NO selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 9;

(3) DNAs according to the above (1) comprising a nucleotide sequence represented by SEQ ID NO: 1 or SEQ ID NO: 2;

(4) DNAs according to the above (1) wherein the DNA is a primer for polymerase chain reaction in order to amplify a DNA coding for a G protein coupled receptor protein;

(5) a method for amplifying a DNA coding for a G

protein coupled receptor protein by polymerase chain reaction techniques, which comprises:

(i) carrying out a polymerase chain reaction in the presence of a mixture of

- ① a DNA coding for G protein coupled receptor protein, said DNA being capable of acting as a template,
- ② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 1, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 3, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 5, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 6, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 7, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 10, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 14, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 16 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 18, and
- ③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 2, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 4, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 8, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 9, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 11, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 15, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 19; or

(ii) carrying out a polymerase chain reaction in the presence of a mixture of

- ① a DNA coding for G protein coupled receptor protein, said DNA being capable of acting as a template,

- ② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 1 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 12, and
- ③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 13;

(6) a method for screening a DNA library for a DNA coding for a G protein coupled receptor protein, which comprises:

(i) carrying out a polymerase chain reaction in the presence of a mixture of

① said DNA library,

- ② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 1, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 3, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 5, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 6, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 7, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 10, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 14, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 16 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 18, and
- ③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 2, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 4, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 8, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 9, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 11, DNA primers comprising a nucleotide

sequence represented by SEQ ID NO: 15, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 19,

5 to amplify selectively a template DNA coding for G protein coupled receptor protein, contained in the DNA library; or (ii) carrying out a polymerase chain reaction in the presence of a mixture of

① said DNA library

10 ② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 1 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 12, and

15 ③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 13,

to amplify selectively a DNA coding for G protein coupled receptor protein, contained in the DNA library;

20 (7) a DNA coding for a G protein coupled receptor protein, which is obtained by a method according to the above (5) or (6); and

(8) G protein coupled receptor proteins encoded by a DNA according to the above (7), their peptide segments or
25 fragments and salts thereof.

Another specific aspect of the invention is:

(9) a method for amplifying a DNA coding for G protein coupled receptor protein (e.g. a region corresponding to from the first to sixth membrane-spanning domains of G
30 protein coupled receptor proteins or other domains thereof) by polymerase chain reaction techniques, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

① a DNA coding for G protein coupled receptor protein, said DNA being capable of acting as a template,

35 ② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 1 and DNA primers

comprising a nucleotide sequence represented by SEQ ID NO: 12, and

- ③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 2, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 4, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 8, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 9, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 15, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 19;

(10) a method for amplifying a DNA coding for G protein coupled receptor protein (e.g. a region corresponding to from the first to seventh membrane-spanning domains of G protein coupled receptor proteins or other domains thereof) by polymerase chain reaction techniques, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

- ① a DNA coding for G protein coupled receptor protein, said DNA being capable of acting as a template,
② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 1 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 12, and

- ③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 11;

(11) a method for amplifying a DNA coding for G protein coupled receptor protein (e.g. a region corresponding to from the third to sixth membrane-spanning domains of G protein coupled receptor proteins or other domains thereof) by polymerase chain reaction techniques, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

- ① a DNA coding for G protein coupled receptor protein,

- said DNA being capable of acting as a template,
- ② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 3, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 5, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 6, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 7, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 14 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 18, and
- ③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 2, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 4, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 8, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 9, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 15, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 19;

(12) a method for amplifying a DNA coding for G protein coupled receptor protein (e.g. a region corresponding to from the third to seventh membrane-spanning domains of G protein coupled receptor proteins or other domains thereof) by polymerase chain reaction techniques, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

- ① a DNA coding for G protein coupled receptor protein, said DNA being capable of acting as a template,
- ② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 3, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 5, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 6, DNA primers comprising a

nucleotide sequence represented by SEQ ID NO: 7, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 14 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 18, and

- 5 ③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 11;

(13) a method for amplifying a DNA coding for G protein coupled receptor protein (e.g. a region corresponding to from the second to sixth membrane-spanning domains of G protein coupled receptor proteins or other domains thereof) by polymerase chain reaction techniques, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

- 10 ① a DNA coding for G protein coupled receptor protein, said DNA being capable of acting as a template,
- 15 ② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 10 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 16, and
- 20 ③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 2, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 4, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 8, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 9, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 15, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 19;
- 25
- 30

(14) a method for amplifying a DNA coding for G protein coupled receptor protein (e.g. a region corresponding to from the second to seventh membrane-spanning domains of G protein coupled receptor proteins or other domains thereof) by polymerase chain reaction techniques, which comprises carrying

35

out a polymerase chain reaction in the presence of a mixture of

- ① a DNA coding for G protein coupled receptor protein, said DNA being capable of acting as a template,
- ② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 10 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 16, and
- ③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 11;

(15) a method for amplifying a DNA coding for G protein coupled receptor protein (e.g., a region corresponding to from the first to third membrane-spanning domains of G protein coupled receptor proteins or other domains thereof) by polymerase chain reaction techniques, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

- ① a DNA coding for G protein coupled receptor protein, said DNA being capable of acting as a template,
- ② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 1 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 12, and
- ③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 13;

(16) a method for amplifying a DNA coding for G protein coupled receptor protein by polymerase chain reaction techniques, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

- ① a DNA coding for G protein coupled receptor protein, said DNA being capable of acting as a template,
- ② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 1, and
- ③ at least one DNA primer selected from the group

consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 2;

(17) a method for amplifying a DNA coding for G protein coupled receptor protein by polymerase chain reaction techniques, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

- ① a DNA coding for G protein coupled receptor protein, said DNA being capable of acting as a template,
- ② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 3, and
- ③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 4;

(18) a method for amplifying a DNA coding for G protein coupled receptor protein by polymerase chain reaction techniques, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

- ① a DNA coding for G protein coupled receptor protein, said DNA being capable of acting as a template,
- ② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 6, and
- ③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 8;

(19) a method for amplifying a DNA coding for G protein coupled receptor protein by polymerase chain reaction techniques, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

- ① a DNA coding for G protein coupled receptor protein, said DNA being capable of acting as a template,
- ② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 10, and
- ③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide

sequence represented by SEQ ID NO: 11;

(20) a method for amplifying DNA coding for a G protein coupled receptor protein which comprises

(i) carrying out a polymerase chain reaction in the presence of a mixture of

- ① a DNA coding for G protein coupled receptor protein, said DNA being capable of acting as a template,
- ② at least one DNA primer which is capable of binding with the 3'-side nucleotide sequence of the - chain (minus chain) of the template DNA coding for G protein coupled receptor protein to allow the extension of the + chain (plus chain) in the 5' → 3' direction, said DNA primer being selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 1, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 3, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 5, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 6, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 7, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 10, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 12, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 14, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 16 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 18, and
- ③ at least one DNA primer which is capable of binding with the 3'-side nucleotide sequence of the + chain (plus chain) of the template DNA coding for G protein coupled receptor protein to allow the extension of the - chain (minus chain) in the 5' → 3' direction, said DNA primer being selected from the group consisting of DNA primers

comprising a nucleotide sequence represented by SEQ ID
NO: 2, DNA primers comprising a nucleotide sequence
represented by SEQ ID NO: 4, DNA primers comprising a
nucleotide sequence represented by SEQ ID NO: 8, DNA
primers comprising a nucleotide sequence represented by
SEQ ID NO: 9, DNA primers comprising a nucleotide
sequence represented by SEQ ID NO: 11, DNA
primers comprising a nucleotide sequence
represented by SEQ ID NO: 15, DNA primers
comprising a nucleotide sequence represented by
SEQ ID NO: 17 and DNA primers comprising a
nucleotide sequence represented by SEQ ID NO: 19,
or

(ii) carrying out a polymerase chain reaction in the presence
of a mixture of

- ① a DNA coding for G protein coupled receptor protein,
said DNA being capable of acting as a template,
- ② at least one DNA primer which is capable of
binding with the 3'-side nucleotide sequence of
the - chain (minus chain) of the template DNA
coding for G protein coupled receptor protein to
allow the extension of the + chain (plus chain)
in the 5' → 3' direction, said DNA primer being
selected from the group consisting of DNA primers
comprising a nucleotide sequence represented by SEQ ID
NO: 1 and DNA primers comprising a nucleotide sequence
represented by SEQ ID NO: 12, and
- ③ at least one DNA primer which is capable of
binding with the 3'-side nucleotide sequence of
the + chain (plus chain) of the template DNA
coding for G protein coupled receptor protein to
allow the extension of the - chain (minus chain)
in the 5' → 3' direction, said DNA primer being
selected from the group consisting of DNA primers
comprising a nucleotide sequence represented by SEQ ID
NO: 13;

(21) a method for screening DNA libraries for

a DNA coding for G protein coupled receptor protein (e.g. from the first to sixth membrane-spanning domains or other domains of G protein coupled receptor protein), which comprises carrying out a polymerase chain reaction in the presence of a mixture of

① said DNA library,

② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 1 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 12, and

③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 2, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 4, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 8, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 9, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 15, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 19,

to amplify selectively a template DNA coding for G protein coupled receptor protein (e.g. from the first to sixth membrane-spanning domains or other domains of G protein coupled receptor protein), contained in the DNA library;

(22) a method for screening DNA libraries for a DNA coding for G protein coupled receptor protein (e.g. from the first to seventh membrane-spanning domains or other domains of G protein coupled receptor protein), which comprises carrying out a polymerase chain reaction in the presence of a mixture of

① said DNA library,

② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide

sequence represented by SEQ ID NO: 1 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 12, and

- ③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 11,

to amplify selectively a template DNA coding for G protein coupled receptor protein (e.g. from the first to seventh membrane-spanning domains or other domains of G protein coupled receptor protein), contained in the DNA library;

(23) a method for screening DNA libraries for a DNA coding for G protein coupled receptor protein (e.g. from the third to sixth membrane-spanning domains or other domains of G protein coupled receptor protein), which comprises carrying out a polymerase chain reaction in the presence of a mixture of

- ① said DNA library,

- ② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 3, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 5, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 6, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 7, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 14 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 18, and

- ③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 2, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 4, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 8, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 9, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 15, DNA

primers comprising a nucleotide sequence
represented by SEQ ID NO: 17 and DNA primers
comprising a nucleotide sequence represented by
SEQ ID NO: 19,

5 to amplify selectively a template DNA coding for G protein
coupled receptor protein (e.g. from the third to sixth
membrane-spanning domains or other domains of G protein coupled
receptor protein), contained in the DNA library;

(24) a method for screening DNA libraries for
10 a DNA coding for G protein coupled receptor protein (e.g. from
the third to seventh membrane-spanning domains or other domains
of G protein coupled receptor protein), which comprises
carrying out a polymerase chain reaction in the presence of a
mixture of

15 ① said DNA library,

② at least one DNA primer selected from the group
consisting of DNA primers comprising a nucleotide
sequence represented by SEQ ID NO: 3, DNA primers
comprising a nucleotide sequence represented by
20 SEQ ID NO: 5, DNA primers comprising a nucleotide
sequence represented by SEQ ID NO: 6, DNA primers
comprising a nucleotide sequence represented by
SEQ ID NO: 7, DNA primers comprising a nucleotide
sequence represented by SEQ ID NO: 14 and DNA
25 primers comprising a nucleotide sequence
represented by SEQ ID NO: 18, and

③ at least one DNA primer selected from the group
consisting of DNA primers comprising a nucleotide
sequence represented by SEQ ID NO: 11,
30 to amplify selectively a template DNA coding for G protein
coupled receptor protein (e.g. from the third to seventh
membrane-spanning domains or other domains of G protein coupled
receptor protein), contained in the DNA library;

(25) a method for screening DNA libraries for
35 a DNA coding for G protein coupled receptor protein (e.g. from
the second to sixth membrane-spanning domains or other domains
of G protein coupled receptor protein), which comprises

carrying out a polymerase chain reaction in the presence of a mixture of

- ① said DNA library,
- ② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 10 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 16, and
- ③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 2, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 4, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 8, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 9, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 15, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 19,

to amplify selectively a template DNA coding for G protein coupled receptor protein (e.g. from the second to sixth membrane-spanning domains or other domains of G protein coupled receptor protein), contained in the DNA library;

(26) a method for screening DNA libraries for a DNA coding for G protein coupled receptor protein (e.g. from the second to seventh membrane-spanning domains or other domains of G protein coupled receptor protein), which comprises carrying out a polymerase chain reaction in the presence of a mixture of

- ① said DNA library,
- ② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 10 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 16, and

③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 11, to amplify selectively a template DNA coding for G protein coupled receptor protein (e.g. from the second to seventh membrane-spanning domains or other domains of G protein coupled receptor protein), contained in the DNA library;

(27) a method for screening DNA libraries for a DNA coding for G protein coupled receptor protein (e.g. from the first to third membrane-spanning domains or other domains of G protein coupled receptor protein), which comprises carrying out a polymerase chain reaction in the presence of a mixture of

① said DNA library,

② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 1 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 12, and

③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 13, to amplify selectively a template DNA coding for G protein coupled receptor protein (e.g. from the first to third membrane-spanning domains or other domains of G protein coupled receptor protein), contained in the DNA library;

(28) a method for screening DNA libraries for a DNA coding for G protein coupled receptor protein, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

① said DNA library,

② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 1, and

③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 2,

to amplify selectively the template DNA coding for G protein coupled receptor protein, contained in the DNA library;

(29) a method for screening DNA libraries to detect a DNA coding for G protein coupled receptor protein, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

- ① said DNA library,
- ② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 3, and
- ③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 4,

to amplify selectively a template DNA coding for G protein coupled receptor protein, contained in the DNA library;

(30) a method for screening DNA libraries for a DNA coding for G protein coupled receptor protein, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

- ① said DNA library,
- ② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 6, and
- ③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 8,

to amplify selectively a template DNA coding for G protein coupled receptor protein, contained in the DNA library;

(31) a method for screening DNA libraries for a DNA coding for G protein coupled receptor protein, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

- ① said DNA library,
- ② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 10, and
- ③ at least one DNA primer selected from the group

consisting of DNA primers comprising a nucleotide
sequence represented by SEQ ID NO: 11,
to amplify selectively a template DNA coding for G protein
coupled receptor protein, contained in the DNA library; and

5 (32) a method for screening DNA libraries according
to any of the above (6), and (21) to (31) wherein said DNA
library is derived from an origin selected from the group
consisting of human tissues and human cells. Examples of such
human tissues include adrenal, umbilical cord, brain, tongue,
10 liver, lymph gland, lung, thymus, placenta, peritoneum, retina,
spleen, heart, smooth muscle, intestine, vessel, bone, kidney,
skin, fetus, mammary gland, ovary, testis, pituitary gland,
pancreas, submandibular gland, spine, prostate gland, stomach,
thyroid gland, trachea (windpipe), skeletal muscle, uterus,
15 adipose tissue, urinary bladder, cornea, olfactory bulb, bone
marrow, amnion, etc. Examples of such human cells include
nerve cells, epithelial cells, endothelial cells, leukocytes,
lymphocytes, gliocytes, fibroblasts, keratinized cells,
osteoblasts, osteoclasts, astrocytes, melanocytes, various
20 carcinomas, various sarcomas, various cells derived from
the above-mentioned human tissues.

Yet another aspect of the present invention is a
degenerate deoxynucleotide which has an oligonucleotide
sequence to which a SEQ ID NO selected from the group
25 consisting of SEQ ID NO: 1 to SEQ ID NO: 19 is assigned.

Another aspect of the present invention is

(33) a G protein coupled receptor protein comprising
an amino acid sequence selected from the group consisting of
amino acid sequences represented by SEQ ID NO: 24 and/or SEQ ID
30 NO: 25 and substantial equivalents to the amino acid
sequence represented by SEQ ID NO: 24 or SEQ ID NO: 25; or
a salt thereof;

(34) a G protein coupled receptor protein according
to the above (33) comprising an amino acid sequence selected
35 from the group consisting of an amino acid sequence represented
by SEQ ID NO: 26 and substantial equivalents to the amino

acid sequence represented by SEQ ID NO: 26; or a salt thereof;

(35) a G protein coupled receptor protein comprising an amino acid sequence selected from the group consisting of an amino acid sequence represented by SEQ ID NO: 27 and

5 substantial equivalents to the amino acid sequence represented by SEQ ID NO: 27; or a salt thereof;

(36) a G protein coupled receptor protein comprising an amino acid sequence selected from the group consisting of an amino acid sequence represented by SEQ ID NO: 28 and

10 substantial equivalents to the amino acid sequence represented by SEQ ID NO: 28; or a salt thereof;

(37) a G protein coupled receptor protein comprising an amino acid sequence selected from the group consisting of amino acid sequences represented by SEQ ID NO: 34 and/or SEQ ID

15 NO: 35 and substantial equivalents to the amino acid sequence represented by SEQ ID NO: 34 or SEQ ID NO: 35; or a salt thereof;

(38) a G protein coupled receptor protein comprising an amino acid sequence selected from the group consisting of an amino acid sequence represented by SEQ ID NO: 38 and

20 substantial equivalents to the amino acid sequence represented by SEQ ID NO: 38; or a salt thereof;

(39) a G protein coupled receptor protein according to the above (38) comprising an amino acid sequence selected

25 from the group consisting of an amino acid sequence represented by SEQ ID NO: 39 and substantial equivalents to the amino acid sequence represented by SEQ ID NO: 39; or a salt thereof;

(40) a G protein coupled receptor protein comprising an amino acid sequence represented by SEQ ID NO: 56 and

30 substantial equivalents to the amino acid sequence represented by SEQ ID NO: 56; or a salt thereof;

(41) a peptide segment or fragment of a G protein coupled receptor protein according to any of the above (33) to (40), a modified derivative thereof or a salt thereof;

35 (42) a DNA which comprises a nucleotide sequence coding for a G protein coupled receptor protein of the above (33);

(43) a DNA which comprises a nucleotide sequence coding for a G protein coupled receptor protein of the above (34);

5 (44) a DNA which comprises a nucleotide sequence coding for a G protein coupled receptor protein of the above (35);

(45) a DNA which comprises a nucleotide sequence coding for a G protein coupled receptor protein of the above (36);

10 (46) a DNA which comprises a nucleotide sequence coding for a G protein coupled receptor protein of the above (37);

15 (47) a DNA which comprises a nucleotide sequence coding for a G protein coupled receptor protein of the above (38);

(48) a DNA which comprises a nucleotide sequence coding for a G protein coupled receptor protein of the above (39);

20 (49) a DNA which comprises a nucleotide sequence coding for a G protein coupled receptor protein of the above (40);

(50) a DNA of the above (42) comprising a nucleotide sequence represented by SEQ ID NO: 29 and/or SEQ ID NO: 30;

25 (51) a DNA of the above (43) comprising a nucleotide sequence represented by SEQ ID NO: 31;

(52) a DNA of the above (44) comprising a nucleotide sequence represented by SEQ ID NO: 32;

(53) a DNA of the above (45) comprising a nucleotide sequence represented by SEQ ID NO: 33;

30 (54) a DNA of the above (46) comprising a nucleotide sequence represented by SEQ ID NO: 36 and/or SEQ ID NO: 37;

(55) a DNA of the above (47) comprising a nucleotide sequence represented by SEQ ID NO: 40;

35 (56) a DNA of the above (48) comprising a nucleotide sequence represented by SEQ ID NO: 41;

(57) a DNA of the above (49) comprising a nucleotide sequence represented by SEQ ID NO: 57;

(58) a vector comprising a DNA according to any of the above (42) to (57);

(59) a transformant (including a transfectant) carrying a vector of the above (58);

5 (60) a process for producing a G protein coupled receptor protein or a salt thereof according to any of the above (33) to (40), which comprises culturing a transformant of the above (59) to express said G protein coupled receptor protein on the membrane of the transformant;

10 (61) a method for determining a ligand to a G protein coupled receptor protein according to any of the above (33) to (40), which comprises contacting

15 (i) at least one component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above (33) to (40), peptide fragments or segments or salts thereof according to the above (41), and mixtures thereof,

with

20 (ii) at least one compound to be tested;

(62) a screening method for a compound capable of inhibiting the binding of a G protein coupled receptor protein according to any of the above (33) to (40) with a ligand, which comprises carrying out a comparison between:

25 (i) at least one case where said ligand is contacted with at least one component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above (33) to (40), peptide fragments or segments or salts thereof
30 according to the above (41), and mixtures thereof,

and

35 (ii) at least one case where said ligand together with a compound to be tested is contacted with at least one component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above (33) to (40), peptide fragments or segments or salts thereof according to the

above (41), and mixtures thereof;

(63) a kit for the screening of one or more compounds capable of inhibiting the binding of a G protein coupled receptor protein according to any of the above (33) to (40), with a ligand, which comprises at least one component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above (33) to (40), peptide fragments or segments or salts thereof according to the above (41), and mixtures thereof; and

(64) an antibody against at least one component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above (33) to (40), peptide fragments or segments or salts thereof according to the above (41), and mixtures thereof.

Yet another aspect of the present invention is

(65) a G protein coupled receptor protein according to the above (33) comprising

(i) an amino acid sequence selected from the group consisting of an amino acid sequence represented by SEQ ID NO: 24, amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are deleted from the amino acid sequence of SEQ ID NO: 24, amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are added to the amino acid sequence of SEQ ID NO: 24, and amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) in the amino acid sequence of SEQ ID NO: 24 are substituted with one or more other amino acid residues, or/and

(ii) an amino acid sequence selected from the group consisting of an amino acid sequence represented by SEQ ID NO: 25, amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are deleted from the amino acid sequence of SEQ ID NO: 25, amino acid

sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are added to the amino acid sequence of SEQ ID NO: 25, and amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) in the amino acid sequence of SEQ ID NO: 25 are substituted with one or more other amino acid residues, or a salt thereof;

(66) a G protein coupled receptor protein according to the above (34) comprising an amino acid sequence selected from the group consisting of an amino acid sequence represented by SEQ ID NO: 26, amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are deleted from the amino acid sequence of SEQ ID NO: 26, amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are added to the amino acid sequence of SEQ ID NO: 26, and amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) in the amino acid sequence of SEQ ID NO: 26 are substituted with one or more other amino acid residues, or a salt thereof;

(67) a G protein coupled receptor protein according to the above (35) comprising an amino acid sequence selected from the group consisting of an amino acid sequence represented by SEQ ID NO: 27, amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are deleted from the amino acid sequence of SEQ ID NO: 27, amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are added to the amino acid sequence of SEQ ID NO: 27, and amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) in

the amino acid sequence of SEQ ID NO: 27 are substituted with one or more other amino acid residues, or a salt thereof;

(68) a G protein coupled receptor protein according to the above (36) comprising an amino acid sequence selected from the group consisting of an amino acid sequence represented by SEQ ID NO: 28, amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are deleted from the amino acid sequence of SEQ ID NO: 28, amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are added to the amino acid sequence of SEQ ID NO: 28, and amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) in the amino acid sequence of SEQ ID NO: 28 are substituted with one or more other amino acid residues, or a salt thereof;

(69) a G protein coupled receptor protein according to the above (37) comprising

(i) an amino acid sequence selected from the group consisting of an amino acid sequence represented by SEQ ID NO: 34, amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are deleted from the amino acid sequence of SEQ ID NO: 34, amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are added to the amino acid sequence of SEQ ID NO: 34, and amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) in the amino acid sequence of SEQ ID NO: 34 are substituted with one or more other amino acid residues, or/and

(ii) an amino acid sequence selected from the group consisting of an amino acid sequence represented by SEQ ID NO: 35, amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more

preferably from 2 to 10 amino acid residues) are deleted from the amino acid sequence of SEQ ID NO: 35, amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are added to the amino acid sequence of SEQ ID NO: 35, and amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) in the amino acid sequence of SEQ ID NO: 35 are substituted with one or more other amino acid residues, or a salt thereof;

(70) a G protein coupled receptor protein according to the above (38) comprising an amino acid sequence selected from the group consisting of an amino acid sequence represented by SEQ ID NO: 38, amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are deleted from the amino acid sequence of SEQ ID NO: 38, amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are added to the amino acid sequence of SEQ ID NO: 38, and amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) in the amino acid sequence of SEQ ID NO: 38 are substituted with one or more other amino acid residues, or a salt thereof;

(71) a G protein coupled receptor protein according to the above (39) comprising an amino acid sequence selected from the group consisting of an amino acid sequence represented by SEQ ID NO: 39, amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are deleted from the amino acid sequence of SEQ ID NO: 39, amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are added to the amino acid sequence of SEQ ID NO: 39, and amino acid sequences wherein one

or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) in the amino acid sequence of SEQ ID NO: 39 are substituted with one or more other amino acid residues, or a salt thereof;

5 (72) a G protein coupled receptor protein according to the above (40) comprising an amino acid sequence selected from the group consisting of an amino acid sequence represented by SEQ ID NO: 56, amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid
10 residues, more preferably from 2 to 10 amino acid residues) are deleted from the amino acid sequence of SEQ ID NO: 56, amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are added to the amino acid
15 sequence of SEQ ID NO: 56, and amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) in the amino acid sequence of SEQ ID NO: 56 are substituted with one or more other amino acid residues, or a salt thereof;

20 (73) a method for determining a ligand according to the above (61) wherein said ligand is selected from the group consisting of angiotensin, bombesin, canavaninoid, cholecystokinin, glutamine, serotonin, melatonin, neuropeptide Y, opioid, purine, vasopressin, oxytocin, VIP (vasoactive
25 intestinal and related peptides), somatostatin, dopamine, motilin, amylin, bradykinin, CGRP (calcitonin gene related peptides), adrenomedullin, leukotriene, pancreastatin, prostaglandin, thromboxanes, adenosine, adrenaline, α - and β -chemokine (IL-8, GRO α , GRO β , GRO γ , NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, MIP1 α ,
30 MIP-1 β , RANTES, etc.), endothelin, enterogastrin, histamine, neurotensin, TRH, pancreatic polypeptides and galanin;

 (74) a method for the screening of a compound or a salt thereof capable of inhibiting the binding of a ligand with
35 a G protein coupled receptor protein according to any of the above (33) to (40), which comprises measuring amounts of a labeled ligand bound to the said G protein coupled receptor

protein in at least two cases:

(i) where the labeled ligand is contacted with at least one component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above (33) to (40), peptide fragments or segments or salts thereof according to the above (41), and mixtures thereof, and

(ii) where the labeled ligand together with a compound to be tested is contacted with at least one component elected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above (33) to (40), peptide fragments or segments or salts thereof according to the above (41), and mixtures thereof,

and comparing the measured amounts of the labeled ligand;

(75) a method for the screening of a compound or a salt thereof capable of inhibiting the binding of a ligand with a G protein coupled receptor protein according to any of the above (33) to (40), which comprises measuring amounts of a labeled ligand bound to a cell comprising the said G protein coupled receptor protein in at least two cases:

(i) where the labeled ligand is contacted with the said cell, and

(ii) where the labeled ligand together with a compound to be tested is contacted with the said cell, and comparing the measured amounts of the labeled ligand;

(76) a method for the screening of a compound or a salt thereof capable of inhibiting the binding of a ligand with a G protein coupled receptor protein according to any of the above (33) to (40), which comprises measuring amounts of a labeled ligand bound to a membrane fraction of a cell comprising the said G protein coupled receptor protein in at least two cases:

(i) where the labeled ligand is contacted with the said membrane fraction, and

(ii) where the labeled ligand together with a compound to be tested is contacted with the membrane fraction,

and comparing the measured amounts of the labeled ligand;

(77) a method for the screening of a compound or a salt thereof capable of inhibiting the binding of a ligand with a G protein coupled receptor protein according to any of the above (33) to (40), which comprises measuring amounts of a labeled ligand bound to said G protein coupled receptor protein in at least two cases:

(i) where the labeled ligand is contacted with a G protein coupled receptor protein according to any of the above (33) to (40) which is expressed on the membrane of a transformant according to the above (59) during incubation of the transformant, and

(ii) where the labeled ligand together with a compound to be tested is contacted with the G protein coupled receptor protein according to any of the above (33) to (40) which is expressed on the membrane of a transformant according to the above (59) during incubation of the transformant,

and comparing the measured amounts of the labeled ligand;

(78) a method for the screening of a compound or a salt thereof capable of inhibiting the binding of a ligand with a G protein coupled receptor protein according to any of the above (33) to (40), which comprises measuring G protein coupled receptor protein-mediated cell-stimulating activities in at least two cases:

(i) where a compound capable of activating the G protein coupled receptor protein according to any of the above (33) to (40) is contacted with a cell comprising the said G protein coupled receptor protein, and

(ii) where the compound capable of activating the G protein together with a compound to be tested is contacted with the cell comprising the said G protein coupled receptor protein,

and comparing the measured cell-stimulating activities;

(79) a method for the screening of a compound or a salt thereof capable of inhibiting the binding of a ligand with a G protein coupled receptor protein according to any of the

above (33) to (40), which comprises measuring G protein coupled receptor protein-mediated cell-stimulating activities in at least two cases:

(i) where a compound capable of activating the G protein coupled receptor protein according to any of the above (33) to (40) is contacted with a G protein coupled receptor protein according to any of the above (33) to (40) which is expressed on the membrane of a transformant according to the above (59) during incubation of the transformant, and

(ii) where the compound capable of activating the G protein together with a compound to be tested is contacted with the G protein coupled receptor protein according to any of the above (33) to (40) which is expressed on the membrane of a transformant according to the above (59) during incubation of the transformant,

and comparing the measured cell-stimulating activities;

(80) a method according to the above (78) or (79) wherein said compound capable of activating the G protein coupled receptor protein according to any of the above (33) to (40) is selected from the group consisting of angiotensin, bombesin, canavaninoid, cholecystokinin, glutamine, serotonin, melatonin, neuropeptide Y, opioid, purine, vasopressin, oxytocin, VIP (vasoactive intestinal and related peptides), somatostatin, dopamine, motilin, amylin, bradykinin, CGRP (calcitonin gene related peptides), adrenomedullin, leukotriene, pancreastatin, prostaglandin, thromboxane, adenosine, adrenaline, α - and β -chemokine (IL-8, GRO α , GRO β , GRO γ , NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, MIP1 α , MIP-1 β , RANTES, etc.), endothelin, enterogastrin, histamine, neurotensin, TRH, pancreatic polypeptides and galanin;

(81) a compound which is determined through a method according to any of the above (62) and (74) to (80) or a salt thereof;

(82) a pharmaceutical composition comprising an

effective amount of a compound according to the above (81) or a salt thereof;

5 (83) a screening kit according to the above (63), comprising a cell comprising a G protein coupled receptor protein according to any of the above (33) to (40);

(84) a screening kit according to the above (63), comprising a membrane fraction derived from a cell comprising a G protein coupled receptor protein according to any of the above (33) to (40);

10 (85) a screening kit according to the above (63), comprising a cell of the (59) or (109) mentioned herein below;

(86) a screening kit according to the above (63), comprising a membrane fraction derived from a cell of the (59) or (109);

15 (87) a compound which is determined by means of a screening kit according to any of the above (63) and (83) to (86) or a salt thereof;

(88) a pharmaceutical composition comprising an effective amount of a compound according to the above (87) or a salt thereof; and

20 (89) a method for measuring at least one component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above (33) to (40), peptide fragments or segments or salts thereof according to the above (41), and mixtures thereof, which comprises contacting an antibody according to the above (64) with the component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above (33) to (40), peptide segments or salts thereof according to the above (41), and mixtures thereof.

Still another aspect of the present invention is

(90) a ligand to a G protein coupled receptor protein according to any of the above (33) to (40), which is determined through the following step of:

35 contacting (i) at least one component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above

(33) to (40), peptide fragments or segments or salts thereof according to the above (41), and mixtures thereof,

with (ii) at least one compound to be examined; and

5 (91) a compound capable of inhibiting the binding of a G protein coupled receptor protein according to any of the above (33) to (40) with a ligand, which is determined through carrying out a comparison between:

10 (i) at least one case where said ligand is contacted with at least one component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above (33) to (40), peptide fragments or segments or salts thereof according to the above (41), and mixtures thereof, and

15 (ii) at least one case where said ligand together with a compound to be tested is contacted with at least one component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above (33) to (40), peptide fragments or segments or salts thereof according to the above (41), and mixtures thereof.

Another aspect of the present invention is

20 (92) a recombinant G protein coupled receptor protein and a salt thereof which is obtained by the expression of a DNA according to any of the above (42) to (57), or a modified or fragmented derivative thereof;

25 (93) a method for amplifying a DNA coding for G protein coupled receptor protein by polymerase chain reaction techniques, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

30 (1) a DNA coding for G protein coupled receptor protein, said DNA being capable of acting as a template, and

35 (2) at least one DNA primer selected from the group consisting of DNA primers comprising either SEQ ID NO: 1 or SEQ ID NO: 2; and

(94) a method for screening DNA libraries for

a DNA coding for G protein coupled receptor protein, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

(1) said DNA library, and

5 (2) at least one DNA primer selected from the group consisting of DNA primers comprising either SEQ ID NO: 1 or SEQ ID NO: 2,

to amplify selectively the DNA coding for G protein coupled receptor protein, contained in the DNA library.

10 Yet another aspect of the present invention is

(95) a monoclonal antibody against at least one component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above (33) to (40), peptide fragments or segments or salts thereof according to the above (41), and mixtures thereof;

15 (96) a preparation of purified polyclonal antibodies against at least one component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above (33) to (40), peptide fragments or segments or salts thereof according to the above (41), and mixtures thereof;

20 (97) an immunoassay for detecting a G protein coupled receptor protein which comprising

(i) incubating a sample to be tested with an antibody according to the above (64) to allow formation of an antigen-antibody complex; and

(ii) detecting an antigen-antibody complex formed in step (i); and

30 (98) an immunoassay for detecting antibodies against a G protein coupled receptor protein which comprising

(i) incubating a sample to be tested with at least one component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above (33) to (40), peptide fragments or segments or salts thereof according to the above (41), and mixtures thereof to allow formation of an antigen-antibody complex; and

(ii) detecting an antigen-antibody complex formed in step (a).

Still another aspect of the present invention is

(99) an antisense DNA or RNA which comprises a nucleotide sequence complementary to at least a portion of a DNA according to any of the above (42) to (57), said antisense DNA or RNA being hybridizable to said DNA according to any of the above (42) to (57);

(100) an antisense DNA or RNA according to the above (99) wherein said antisense DNA or RNA comprises the 5' end hairpin loop, 5' end 6-base-pair repeat, 5' end untranslated region, protein translation initiation site or codon, ORF translation initiation site or codon, 3'-untranslated region, 3' end palindrome region, or 3' end hairpin loop of a G protein coupled receptor protein DNA according to any of the above (42) to (57);

(101) an antisense DNA or RNA according to the above (99) in a pharmaceutically acceptable carrier;

(102) an antisense DNA or RNA according to the above (99) comprising from 2 to 50 nucleotides;

(103) a method for modulating the activity of a G protein coupled receptor protein comprising contacting cells expressing the G protein coupled receptor protein with an antisense DNA or RNA according to the above (99);

(104) a method for producing an antibody against a G protein coupled receptor protein according to any of the above (33) to (40), which comprises administering to an individual at least one component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above (33) to (40), peptide fragments or segments or salts thereof according to the above (41), and mixtures thereof; and

(105) a method for producing a hybridoma which produces a monoclonal antibody against a G protein coupled receptor protein according to any of the above (33) to (40), which comprises

(i) immunizing an individual with at least one

component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above (33) to (40), peptide fragments or segments or salts thereof according to the above (41), and mixtures thereof;

5 (ii) immortalizing antibody producing cells from the immunized individual;

(iii) selecting an immortal cell which produces antibodies reactive with the G protein coupled receptor protein; and

10 (iv) growing said immortal cell.

Yet another aspect of the present invention is

(106) a PCR screening kit for a DNA (or nucleotide sequence) coding for G protein coupled receptor protein in a DNA library which comprises

- 15 (i) ① at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 1, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 3, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 5, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 6, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 7, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 10, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 14, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 16 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 18, and
- 20 ② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 2, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 4, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 8, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 9, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 11, DNA primers comprising a nucleotide
- 25
- 30
- 35

sequence represented by SEQ ID NO: 15, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 19; or

5 (ii)① at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 1 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 12, and

10 ② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 13;

(107) a vector comprising the DNA according to the above (7);

15 (108) an expression system comprising an open reading frame (ORF) of DNA derived from a G protein coupled receptor protein DNA according to any of the above (7) and (42) to (57), wherein the ORF is operably linked to a control sequence compatible with a desired host cell;

20 (109) a transformant (including a transfectant) carrying a vector of the above (107) or an expression system of the above (108);

(110) a process for producing a G protein coupled receptor protein or a salt thereof, which comprises culturing the transformant of the above (109) to express said G protein coupled receptor protein on the membrane of the transformant;

25 (111) a method for expressing a polypeptide of G protein coupled receptor protein, comprising:

30 (a) providing a transformant of the above (59) or (109); and

(b) incubating the transformant under conditions which allow expression of the polypeptide of G protein coupled receptor protein;

(112) a method for preparing a transformant according to the above (59) or (109), comprising:

35 (a) providing a host cell capable of transformation;

(b) providing a vector according to the above (58) or (107) or an expression system according to the above (108); and

(c) incubating (a) with (b) under conditions which allow transformation of the host cell with the vector or the expression system;

(113) a pharmaceutical composition according to the above (82) or (88), comprising an effective amount of a compound according to the above (81) or (87) or a pharmaceutically acceptable salt thereof in admixture with a pharmaceutically acceptable carrier, excipient or diluent;

(114) the pharmaceutical composition according to the above (82) or (88), for inhibiting the binding of a G protein coupled receptor protein according to the present invention with a ligand;

(115) a method for inhibiting the binding of a G protein coupled receptor protein according to the present invention with a ligand in a medium which comprises contacting an effective amount of a compound according to the above (81) or (87) or a salt thereof with said medium;

(116) a method for modulating the activity of a G protein coupled receptor protein comprising contacting cells expressing the G protein coupled receptor protein with an effective amount of a compound according to the above (81) or (87) or a salt thereof;

(117) the ligand according to the above (90) being labeled with a detectable reporter;

(118) the antibody according to the above (64) wherein the antibody is labeled with a detectable reporter;

(119) a pharmaceutical composition for controlling an expression of G protein coupled receptor protein, which comprises an effective amount of the antisense DNA according to the above (99), and

(120) a culture product produced by a transformant according to the above (59) or (109).

Yet another aspect of the present invention is

(121) a DNA according to the above (1) wherein the

DNA is an oligonucleotide having from 8 to 60 base residues;

(122) a DNA according to the above (1) wherein the DNA is synthetic;

5 (123) a DNA (or nucleotide sequence) coding for a G protein coupled receptor protein or a fragment thereof, which is obtained through the method according to any of the above (5) to (32);

10 (124) a DNA (or nucleotide sequence) according to the above (123), wherein said G protein coupled receptor protein is selected from the group consisting of angiotensin receptor, bombesin receptor, canavaninoid receptor, cholecystokinin receptor, glutamine receptor, serotonin receptor, melatonin receptor, neuropeptide Y receptor, opioid receptor, purine receptor, vasopressin receptor, oxytocin
15 receptor, VIP receptor (vasoactive intestinal and related peptide receptor), somatostatin receptor, dopamine receptor, motilin receptor, amylin receptor, bradykinin receptor, CGRP receptor (calcitonin gene related peptide receptor), adrenomedullin receptor, leukotriene receptor, pancreastatin
20 receptor, prostaglandin receptor, thromboxane receptor, adenosine receptor, adrenaline receptor, α - and β -chemokine receptor including IL-8, GRO α , GRO β , GRO γ , NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, MIP1 α , MIP-1 β , and RANTES receptors, endothelin receptor, enterogastrin
25 receptor, histamine receptor, neurotensin receptor, TRH receptor, pancreatic polypeptide receptor, and galanin receptor; and

(125) a culture product produced by a transformant according to the above (59) or (109).

30 As used herein the term "substantial equivalent(s)" means that the activity of the protein, e.g., nature of the ligand binding activity, and physical characteristics are substantially the same. Substitutions, deletions or
35 insertions of amino acids often do not produce radical changes in the physical and chemical characteristics of a polypeptide, in which case polypeptides containing the substitution,

deletion, or insertion would be considered to be substantially equivalent to polypeptides lacking the substitution, deletion, or insertion. Substantially equivalent substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs.

The non-polar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts the community (homology) of the sequence of 5' side synthetic DNA primers (HS-1) having a nucleotide sequence represented by SEQ ID NO: 1 with the nucleotide sequence each of other G protein coupled receptor protein-encoding cDNAs and genes.

Figure 2 depicts the community (homology) of the sequence which is complementary to 3' side synthetic DNA primers (HS-2) having a nucleotide sequence represented by SEQ ID NO: 2 with the nucleotide sequence each of other G protein coupled receptor protein-encoding cDNAs and genes.

Figure 3 depicts the community (homology) of the sequence of 5' side synthetic DNA primers (3A) having a nucleotide sequence represented by SEQ ID NO: 5 or 5' side synthetic DNA primers (3B) having a nucleotide sequence represented by SEQ ID NO: 6 relative to the nucleotide sequence each of other G protein coupled receptor protein-encoding cDNAs and genes.

Figure 4 depicts the community (homology) of the sequence of 5' side synthetic DNA primers (3C) having a nucleotide sequence represented by SEQ ID NO: 7 or 5' side synthetic DNA primers (3D) having a nucleotide sequence represented by SEQ ID NO: 3 relative to the nucleotide sequence each of other G protein coupled receptor protein-encoding cDNAs

and genes.

Figure 5 depicts the community (homology) of the sequence (6A) which is complementary to 3' side synthetic DNA primers having a nucleotide sequence represented by SEQ ID NO: 8 or the nucleotide sequence (6B) which is complementary to 3' side synthetic DNA primers having a nucleotide sequence represented by SEQ ID NO: 9 relative to the nucleotide sequence each of other G protein coupled receptor protein-encoding cDNAs and genes.

Figure 6 depicts the community (homology) of the sequence (6C) which is complementary to 3' side synthetic DNA primers having a nucleotide sequence represented by SEQ ID NO: 4 relative to the nucleotide sequence each of other G protein coupled receptor protein-encoding cDNAs and genes.

Figure 7 depicts the community (homology) of the sequence of 5' side synthetic DNA primers (T2A) having a nucleotide sequence represented by SEQ ID NO: 10 with the nucleotide sequence each of other G protein coupled receptor protein-encoding cDNAs and genes.

Figure 8 depicts the community (homology) of the sequence which is complementary to 3' side synthetic DNA primers (T7A) having a nucleotide sequence represented by SEQ ID NO: 11 relative to the nucleotide sequence each of other G protein coupled receptor protein-encoding cDNAs and genes.

Figure 9 depicts the community (homology) of the sequence of 5' side synthetic DNA primers (TM1-A2) having a nucleotide sequence represented by SEQ ID NO: 12 relative to the nucleotide sequence each of other G protein coupled receptor protein-encoding cDNAs and genes.

Figure 10 depicts the community (homology) of the sequence which is complementary to 3' side synthetic DNA primers (TM3-B2) having a nucleotide sequence represented by SEQ ID NO: 13 relative to the nucleotide sequence each of other G protein coupled receptor protein-encoding cDNAs and genes.

Figure 11 depicts the community (homology) of the sequence of 5' side synthetic DNA primers (TM3-C2) having a nucleotide sequence represented by SEQ ID NO: 14 relative to

the nucleotide sequence each of other G protein coupled receptor protein-encoding cDNAs and genes.

Figure 12 depicts the community (homology) of the sequence which is complementary to 3' side synthetic DNA primers (TM6-E2) having a nucleotide sequence represented by SEQ ID NO: 15 relative to the nucleotide sequence each of other G protein coupled receptor protein-encoding cDNAs and genes.

Figure 13 depicts the community (homology) of the sequence of 5' side synthetic DNA primers (TM2F18) having a nucleotide sequence represented by SEQ ID NO: 16 relative to the nucleotide sequence each of other G protein coupled receptor protein-encoding cDNAs and genes.

Figure 14 depicts the community (homology) of the sequence which is complementary to 3' side synthetic DNA primers (TM6R21) having a nucleotide sequence represented by SEQ ID NO: 17 relative to the nucleotide sequence each of other G protein coupled receptor protein-encoding cDNAs and genes.

Figure 15 depicts the community (homology) of the sequence of 5' side synthetic DNA primers (S3A) having a nucleotide sequence represented by SEQ ID NO: 18 relative to the nucleotide sequence each of other G protein coupled receptor protein-encoding cDNAs and genes.

Figure 16 depicts the community (homology) of the sequence which is complementary to 3' side synthetic DNA primers (S6A) having a nucleotide sequence represented by SEQ ID NO: 19 relative to the nucleotide sequence each of other G protein coupled receptor protein-encoding cDNAs and genes.

Figure 17 is the 1.2% agarose gel electrophoresis profile of cDNA products each obtained from human brain amygdala (1, 2, 7), human pituitary body (3, 4, 8) and rat brain (5, 6, 9) by PCR amplification using the synthetic DNA primers having a nucleotide sequence represented by SEQ ID NO: 1 and the synthetic DNA primers having a nucleotide sequence represented by SEQ ID NO: 2, wherein lanes 1 to 6 show the results of when PCR is carried out under severe conditions as disclosed in Examples, lanes 7 to 9 show the results of when PCR is carried out under mild conditions, and M denotes a size

marker which is obtained by cutting λ -phage DNA with restriction enzyme, EcoT14I.

Figure 18 shows the nucleotide sequence determined by sequencing of clone A58, with a T7 primer wherein the clone A58 is obtained by amplifying human brain amygdala-derived cDNA by PCR under mild conditions and subcloning it to pCRTM II.

Figure 19 shows the nucleotide sequence determined by sequencing of clone A58 with an SP6 primer.

Figure 20 shows the nucleotide sequence determined by sequencing of clone 57-A-2 by using a -21M13 primer wherein the clone 57-A-2 is obtained by amplifying human brain amygdala-derived cDNA by PCR under severe conditions and subcloning it to pCRTM II.

Figure 21 shows the nucleotide sequence determined by sequencing of clone B54 with a T7 primer wherein the clone B54 is obtained by amplifying rat whole brain-derived cDNA by PCR under mild conditions and subcloning it to pCRTM II.

Figure 22 illustrates the nucleotide sequence of the human pituitary gland-derived G protein coupled receptor protein cDNA fragment included in the cDNA clone p19P2 isolated by PCR using a human pituitary gland-derived cDNA and the amino acid sequence encoded thereby, wherein the primer used for sequencing is -21M13, and the underlined part corresponds to the synthetic primer.

Figure 23 illustrates the nucleotide sequence of the human pituitary gland-derived G protein coupled receptor protein cDNA fragment included in the cDNA clone p19P2 isolated by PCR using a human pituitary gland-derived cDNA and the amino acid sequence encoded thereby, wherein the primer used for sequencing is M13RV-N (Takara, Japan), and the underlined part corresponds to the synthetic primer.

Figure 24 is the partial hydrophobicity plotting profile of the protein encoded by the human pituitary gland-derived G protein coupled receptor protein cDNA fragment included in p19P2, prepared based upon the amino acid sequence

shown in Figure 22.

Figure 25 is the partial hydrophobicity plotting profile of the protein encoded by the human pituitary gland-derived G protein coupled receptor protein cDNA fragment included in p19P2, prepared based upon the amino acid sequence shown in Figure 23.

Figure 26 shows the partial amino acid sequence (p19P2) of the protein encoded by the human pituitary gland-derived G protein coupled receptor protein cDNA fragment included in p19P2, as shown in Figures 22 and 23, relative to the known G protein coupled receptor protein, S12863, wherein reverse amino acid residues are in agreement, the 1st to 99th amino acid residues of the p19P2 sequence correspond to the 1st to 99th amino acid residues in Figure 22, and the 156th to 230th amino acid residues thereof correspond to the 1st to 68th amino acid residues in Figure 23.

Figure 27 is the nucleotide sequence of the MIN6-derived G protein coupled receptor protein cDNA fragment derived based upon the nucleotide sequences of the MIN6-derived G protein coupled receptor protein cDNA fragments each included in the cDNA clones, pG3-2 and pG1-10, isolated by PCR using a MIN6-derived cDNA and the amino acid sequence encoded thereby, wherein the underlined parts corresponds to the synthetic primers.

Figure 28 is the partial hydrophobicity plotting profile of the MIN6-derived G protein coupled receptor protein, prepared based upon the partial amino acid sequence shown in Figure 27.

Figure 29 is the partial nucleotide sequence of the novel receptor protein cDNA clone, p63A2, obtained from the human amygdaloid nucleus by PCR amplification and the amino acid sequence encoded thereby, wherein the underlined part corresponds to the synthetic primer.

Figure 30 is the partial nucleotide sequence of the novel receptor protein cDNA clone, p63A2, obtained from the human amygdaloid nucleus by PCR amplification and the amino acid sequence encoded thereby, wherein the underlined part

corresponds to the synthetic primer.

Figure 31 is the hydrophobicity plotting profile, prepared based upon the amino acid sequence shown in Figure 29, suggesting the presence of hydrophobic domains as designated by 1 to 3.

Figure 32 is the hydrophobicity plotting profile, prepared based upon the amino acid sequence shown in Figure 30, suggesting the presence of hydrophobic domains as designated by 4 to 6.

Figure 33 is the partial amino acid sequence (p63A2) of the protein encoded by the novel receptor protein cDNA fragment included in p63A2, relative to the partial amino acid sequence of the G protein coupled receptor protein (P30731) expressed and induced by a mouse T cell-derived glucocorticoid, wherein reverse amino acid residues are in agreement.

Figure 34 is the whole nucleotide sequence of the the human pituitary gland-derived G protein coupled receptor protein cDNA, included in the cDNA clone, phGR3, isolated from the human-derived cDNA library by plaque hybridization using an DNA insert in the p19P2 as a probe, and the amino acid sequence encoded thereby.

Figure 35 is the northern blotting profile of the human pituitary gland mRNA of the receptor gene encoded by the human pituitary gland-derived cDNA clone, phGR3.

Figure 36 is the hydrophobicity plotting profile of the protein encoded by the human pituitary gland-derived G protein coupled receptor protein cDNA included in phGR3, prepared based upon the amino acid sequence shown in Figure 34.

Figure 37 is the partial nucleotide sequence of the novel receptor protein cDNA clone, p3H2-17, obtained from mouse pancreatic β -cell strain, MIN6, by PCR amplification and the amino acid sequence encoded thereby, wherein the underlined part corresponds to the synthetic primer used for the PCR amplification.

Figure 38 is the hydrophobicity plotting profile, prepared based upon the amino acid sequence shown in Figure 37, suggesting the presence of hydrophobic domains as designated

by 3 to 6.

Figure 39 is the partial amino acid sequence encoded by the novel receptor protein cDNA included in p3H2-17, relative to the partial amino acid sequence each of chicken ATP receptor protein (P34996), human somatostatin receptor subtype 3 protein (A46226), human somatostatin receptor subtype 4 protein (JN0605) and bovine neuropeptide Y receptor protein (S28787), wherein reverse amino acid residues are in agreement.

Figure 40 is the partial nucleotide sequence of the novel receptor protein cDNA clone, p3H2-34, obtained from mouse pancreatic β -cell strain, MIN6, by PCR amplification and the amino acid sequence encoded thereby, wherein the underlined parts correspond to the synthetic primers used for the PCR amplification.

Figure 41 is the hydrophobicity plotting profile, prepared based upon the amino acid sequence shown in Figure 40, wherein the axis of ordinate represents an index of hydrophobicity, the axis of abscissa represents the number of amino acids and numerals 3 to 6 represent the presence of hydrophobic domains.

Figure 42 is the partial amino acid sequence encoded by the novel receptor protein cDNA included in p3H2-34, relative to the partial amino acid sequence each of human somatostatin receptor subtype 4 protein (JN0605), human somatostatin receptor subtype 2 protein (B41795) and rat-derived ligand unknown receptor protein (A39297), wherein reverse amino acid residues are in agreement.

Figure 43 is the nucleotide sequence of the rabbit gastropyloric part smooth muscle-derived G protein coupled receptor protein cDNA fragment included in the novel receptor protein cDNA clone, pMD4, obtained from rabbit gastropyloric part smooth muscles by PCR amplification, and the amino acid sequence encoded thereby, wherein the underlined parts correspond to the synthetic primers used for the PCR amplification.

Figure 44 is the hydrophobicity plotting profile of the protein encoded by the rabbit gastropyloric part smooth

muscle-derived G protein coupled receptor protein cDNA fragment included in pMD4, prepared based upon the amino acid sequence shown in Figure 35, wherein numerals 1 to 3 suggest the presence of hydrophobic domains.

5 Figure 45 is the partial amino acid sequence (pMD4) of the protein encoded by the rabbit gastropyrolic part smooth muscle-derived G protein coupled receptor protein cDNA fragment included in pMD4 as shown in Figure 43, relative to the known G protein coupled receptor protein, rat ligand unknown receptor protein (A35639), wherein reverse amino acid residues are in
10 agreement, the 1st to 88th amino acid residues of the pMD4 sequence correspond to the 1st to 88th amino acid residues in Figure 43.

15 Figure 46 shows the nucleotide sequence of the mouse-derived galanin receptor protein cDNA clone, pMGR20, which has been cloned with, as a probe, the cDNA insert in p3H2-34 and the amino acid sequence encoded thereby.

20 Figure 47 is the hydrophobicity plotting profile, prepared based upon the amino acid sequence shown in Figure 46, wherein the axis of ordinate represents an index of hydrophobic property, the axis of abscissa represents the number of amino acids, and numerals 1 to 7 represent the presence of hydrophobic domains.

25 Figure 48 is the amino acid sequence (MOUSEGALRECE) of the mouse-derived galanin receptor protein encoded by pMGR20, relative to the amino acid sequence (HUMAGALAMI) of the human-derived galanin receptor protein, wherein reverse amino acid residues are in agreement.

30 Figure 49 is the nucleotide sequence of the rabbit gastropyrolic part smooth muscle-derived G protein coupled receptor protein cDNA fragment included in the novel receptor protein cDNA clone, pMJ10, obtained from rabbit gastropyrolic part smooth muscles by PCR amplification and the amino acid sequence encoded thereby, wherein the underlined parts
35 corresponds to the synthetic primers used for the PCR amplification.

Figure 50 is the hydrophobicity plotting profile of

the protein encoded by the rabbit gastropyrolic part smooth muscle-derived G protein coupled receptor protein cDNA fragment included in pMJ10, prepared based upon the amino acid sequence shown in Figure 49, wherein numerals 4 to 6 suggest the presence of hydrophobic domains.

Figure 51 is the partial amino acid sequence (pMJ10) of the protein encoded by the rabbit gastropyrolic part smooth muscle-derived G protein coupled receptor protein cDNA fragment included in pMJ10 shown in Figure 49, relative to human ligand unknown receptor protein (B42009), human N-formylpeptide receptor protein (JC2014), rabbit N-formylpeptide receptor protein (A46520), mouse C5a anaphylatoxin receptor protein (A46525) and bovine neuropeptide Y receptor protein (S28787) which are known G protein coupled receptor proteins, wherein reverse amino acid residues are in agreement, and the 1st to 125th amino acid residues of pMJ10 correspond to the 1st to 125th amino acid residues in Figure 49.

Figure 52 is the nucleotide sequence of the rabbit gastropyrolic part smooth muscle-derived G protein coupled receptor protein cDNA fragment included in the novel receptor protein cDNA clone, pMH28, obtained from rabbit gastropyrolic part smooth muscles by PCR amplification and the amino acid sequence encoded thereby, wherein the underlined parts correspond to the synthetic primers used for the PCR amplification.

Figure 53 is the hydrophobicity plotting profile of the protein encoded by the rabbit gastropyrolic part smooth muscle-derived G protein coupled receptor protein cDNA fragment included in pMH28, prepared based upon the amino acid sequence shown in Figure 52, wherein numerals 4 to 6 suggest the presence of hydrophobic domains.

Figure 54 is the partial amino acid sequence (pMH28) of the protein encoded by the rabbit gastropyrolic part smooth muscle-derived G protein coupled receptor protein cDNA fragment included in pMH28 shown in Figure 52, relative to mouse IL-8 receptor protein (P35343), human somatostatin receptor protein 1 (A41795) and human somatostatin receptor protein 4 (A47457).

which are known G protein coupled receptor proteins, wherein reverse amino acid residues are in agreement, and the 1st to 119th amino acid residues of pMH28 correspond to the 1st to 119th amino acid residues in Figure 52.

5 Figure 55 is the nucleotide sequence, of the rabbit gastropyrolic part smooth muscle-derived G protein coupled receptor protein cDNA fragment included in the novel receptor protein cDNA clone, pMN7, obtained from rabbit gastropyrolic part smooth muscles by PCR amplification and the amino acid
10 sequence encoded thereby, wherein the underlined 5'-end nucleotide sequence part corresponds to the synthetic primer used for the PCR amplification.

15 Figure 56 is the nucleotide sequence of the rabbit gastropyrolic part smooth muscle-derived G protein coupled receptor protein cDNA fragment included in the novel receptor protein cDNA clone, pMN7, obtained from rabbit gastropyrolic part smooth muscles by PCR amplification and the amino acid
20 sequence encoded thereby, wherein the underlined 3'-end nucleotide sequence part corresponds to the synthetic primer used for the PCR amplification.

25 Figure 57 is the hydrophobicity plotting profile of the protein encoded by the rabbit gastropyrolic part smooth muscle-derived G protein coupled receptor protein cDNA fragment included in pMN7, prepared based upon the amino acid sequences shown in Figures 55 and 56, wherein numerals TM2 to TM6 suggest the presence of hydrophobic domains.

30 Figure 58 is the partial hydrophobicity plotting profile of the protein encoded by the human pituitary gland-derived G protein coupled receptor protein cDNA fragment included in p19P2, prepared based upon the amino acid sequence shown in Figure 22.

35 Figure 59 is the partial hydrophobicity plotting profile of the protein encoded by the human pituitary gland-derived G protein coupled receptor protein cDNA fragment included in p19P2, prepared based upon the amino acid sequence shown in Figure 23.

Figure 60 shows the partial amino acid sequence

(p19P2) of the protein encoded by the human pituitary gland-derived G protein coupled receptor protein cDNA fragment included in p19P2, as shown in Figures 22 and 23, relative to the known G protein coupled receptor protein, S12863, wherein reverse amino acid residues are in agreement, the 1st to 99th amino acid residues of the p19P2 sequence correspond to the 1st to 99th amino acid residues in Figure 22, and the 156th to 230th amino acid residues thereof correspond to the 1st to 68th amino acid residues in Figure 23.

Figure 61 is the partial amino acid sequence (pG3-2/pG1-10) of the MIN6-derived G protein coupled receptor protein, as shown in Figure 27, relative to the partial amino acid sequence (p19P2) of the protein encoded by p19P2, as shown in Figures 22 and 23, wherein reverse amino acid residues are in agreement, the 1st to 99th amino acid residues of the p19P2 sequence correspond to the 1st to 99th amino acid residues in Figure 22, the 156th to 223rd amino acid residues thereof correspond to the 1st to 68th amino acid residues in Figure 23, and the 1st to 223rd amino acid residues of the pG3-2/pG1-10 sequence correspond to the 1st to 223rd amino acid residues in Figure 27.

Figure 62 is the nucleotide sequence of the MIN6-derived G protein coupled receptor protein cDNA fragment included in the cDNA clone, p5S38, isolated by PCR using a MIN6-derived cDNA and the amino acid sequence encoded thereby, wherein the underlined parts corresponds to the synthetic primers.

Figure 63 is the partial amino acid sequence (p5S38) of the MIN6-derived G protein coupled receptor protein, as shown in Figure 62, relative to the partial amino acid sequence (p19P2) of the G protein coupled receptor protein encoded by p19P2, as shown in Figures 22 and 23, as well as the partial amino acid sequence of the G protein coupled receptor protein encoded by the nucleotide sequence derived from the nucleotide sequence of the cDNA fragment included in pG3-2 and pG1-10, as shown in Figure 27, wherein reverse amino acid residues are in agreement, the 1st to 144th amino acid residues of the p5S38

sequence correspond to the 1st to 144th amino acid residues in Figure 62, the 1st to 99th amino acid residues of the p19P2 sequence correspond to the 1st to 99th amino acid residues in Figure 22, the 156th to 223rd amino acid residues thereof correspond to the 1st to 68th amino acid residues in Figure 23, and the 1st to 223rd amino acid residues of the pG3-2/pG1-10 sequence correspond to the 1st to 223rd amino acid residues in Figure 27.

Figure 64 is the partial hydrophobicity plotting profile of the protein encoded by the MIN6-derived G protein coupled receptor protein cDNA fragment included in p5S38, prepared based upon the amino acid sequence shown in Figure 62.

Figure 65 shows the northern blot analysis profile of the receptor gene encoded by the cDNA included in the mouse pancreatic β -cell strain MIN6-derived novel receptor protein cDNA clone, p3H2-17, for mouse cell line, MIN6, Neuro-2a cell and mouse brain, thymus, spleen and pancreas poly(A)⁺ RNA, wherein each arrow and number indicates the size marker position (unit of number: kb).

Figure 66 shows the agarose gel electrophoresis analysis profile of the PCR products obtained by 5'RACE PCR of the receptor gene included in p3H2-17 using mouse thymus and spleen poly(A)⁺ RNA.

Lane 1 indicates the size marker 6 (Wako Pure Chemical, Japan).

Lane 2 indicates the internal control which is the thymus-derived PCR product obtained by PCR amplification using the primer having SEQ ID NO: 20 and the primer having SEQ ID NO: 22 with Taq polymerase.

Lane 3 indicates the negative control which is the PCR product obtained by Ex Taq polymerase PCR amplification of thymus cDNA prior to addition of anchors.

Lane 4 indicates the negative control which is the PCR product obtained by Taq polymerase PCR amplification of thymus cDNA prior to addition of anchors.

Lane 5 indicates the PCR product obtained by 5'RACE of thymus poly(A)⁺ RNA with Pfu polymerase.

Lane 6 indicates the PCR product obtained by 5'RACE of thymus poly(A)⁺ RNA with Vent polymerase.

Lane 7 indicates the PCR product obtained by 5'RACE of thymus poly(A)⁺ RNA with Ex Taq polymerase.

5 Lane 8 indicates the PCR product obtained by 5'RACE of thymus poly(A)⁺ RNA with Taq polymerase.

Lane 9 indicates the size marker 5 (Wako Pure Chemical, Japan).

10 Lane 10 indicates the internal control which is the spleen-derived PCR product obtained by PCR amplification using the primer having SEQ ID NO: 20 and the primer having SEQ ID NO: 22 with Taq polymerase.

15 Lane 11 indicates the negative control which is the PCR product obtained by Ex Taq polymerase PCR amplification of spleen cDNA prior to addition of anchors.

Lane 12 indicates the negative control which is the PCR product obtained by Taq polymerase PCR amplification of spleen cDNA prior to addition of anchors.

20 Lane 13 indicates the PCR product obtained by 5'RACE of poly(A) RNA⁺ with Pfu polymerase.

Lane 14 indicates the PCR product obtained by 5'RACE of spleen poly(A)⁺ RNA with Vent polymerase.

Lane 15 indicates the PCR product obtained by 5'RACE of spleen poly(A)⁺ RNA with Ex Taq polymerase.

25 Lane 16 indicates the PCR product obtained by 5'RACE of spleen poly(A)⁺ RNA with Taq polymerase.

Lane 17 indicates the size marker 5 (Wako Pure Chemical, Japan).

Each blacked triangle indicates the band recovered.

30 Figure 67 shows the agarose gel electrophoresis analysis profile of the PCR products obtained by 3'RACE PCR of the receptor gene included in p3H2-17 using mouse thymus and spleen poly(A)⁺ RNA.

35 Lane 1 indicates the size marker 5 (Wako Pure Chemical, Japan).

Lane 2 indicates the PCR product obtained by 3'RACE of spleen poly(A)⁺ RNA with Taq polymerase.

Lane 3 indicates the PCR product obtained by 3'RACE of spleen poly(A)⁺ RNA with Ex Taq polymerase.

Lane 4 indicates the PCR product obtained by 3'RACE of spleen poly(A)⁺ RNA with Vent polymerase.

5 Lane 5 indicates the PCR product obtained by 3'RACE of spleen poly(A)⁺ RNA with Pfu polymerase.

Lane 6 indicates the PCR product obtained by 3'RACE of thymus poly(A)⁺ RNA with Taq polymerase.

10 Lane 7 indicates the PCR product obtained by 3'RACE of thymus poly(A)⁺ RNA with Ex Taq polymerase.

Lane 8 indicates the PCR product obtained by 3'RACE of thymus poly(A)⁺ RNA with Vent polymerase.

Lane 9 indicates the PCR product obtained by 3'RACE of thymus poly(A)⁺ RNA with Pfu polymerase.

15 Lane 10 indicates the size marker 6 (Wako Pure Chemical, Japan).

Each blacked triangle indicates the band recovered.

Figure 68 depicts the model of the RACE products of the receptor protein cDNA fragment included in p3H2-17 obtained by 5'RACE and 3'RACE. Open squares represent regions which have already been isolated and included in p3H2-17. Small arrows, ①, ②, ③ and ④, indicate the positions and directions of the primers designed in Working Example 19. The big arrow shows a predicted full-length open reading frame of the receptor protein held by p3H2-17. Numbers at both ends, N26, N64, N75, C2, C13 and C15, indicate clone numbers of the RACE products obtained. Among these RACE products, N26, N64 and N75 are inserted into pCRTM II vector and C2, C13 and C15 are inserted into the SmaI site of pUC18. The solid triangle indicates the PCR error position which has been clarified through sequencing.

Figure 69 is the nucleotide sequence of the open reading frame and neighboring regions thereof of mouse G protein coupled receptor protein cDNA included in the cDNA clone pMAH2-17 obtained from mouse spleen and thymus poly(A) RNA by RACE techniques based on the nucleotide sequence of the cDNA fragment included in p3H2-17 and the amino acid

sequence encoded thereby.

Figure 70 is the hydrophobicity plotting profile of the protein encoded by the receptor protein cDNA included in pMAH2-17, prepared based upon the amino acid sequence shown in Figure 69.

Figure 71 is the amino acid sequence (75+13CODING) of the protein encoded by the mouse-derived G protein coupled receptor protein cDNA fragment included in pMAH2-17, as shown in Figure 69, relative to the known G protein coupled receptor proteins, mouse P_{2U} purinoceptor (P2UR MOUSE) and chicken P_{2Y} purinoceptor (P2YR CHICK), wherein reverse amino acid residues are in agreement.

Figure 72 is the nucleotide sequence (from 1st to 540th nucleotides) of the rabbit gastropyrolic part smooth muscle-derived G protein coupled receptor protein cDNA fragment included in the novel receptor protein cDNA clone, pMN128, obtained from rabbit gastropyrolic part smooth muscles by PCR amplification, and the amino acid sequence encoded thereby, wherein the underlined 5' part corresponds to the synthetic primer used for the PCR amplification.

Figure 73 is the nucleotide sequence (from 541st to 843rd nucleotides) of the rabbit gastropyrolic part smooth muscle-derived G protein coupled receptor protein cDNA fragment included in the novel receptor protein cDNA clone, pMN128, obtained from rabbit gastropyrolic part smooth muscles by PCR amplification, and the amino acid sequence encoded thereby, wherein the underlined 3' part corresponds to the synthetic primer used for the PCR amplification.

Figure 74 is the hydrophobicity plotting profile of the protein encoded by the rabbit gastropyrolic part smooth muscle-derived G protein coupled receptor protein cDNA fragment included in pMN128, prepared based upon the amino acid sequences shown in Figures 72 and 73, suggesting the presence of hydrophobic domains.

Figure 75 shows inward currents evoked by ATP in Xenopus oocytes injected with cDNA of pMAH2-17-encoded receptor.

Figure 76 is the nucleotide sequence of the human-derived G protein coupled receptor protein cDNA fragment included in ph3H2-17, relative to the nucleotide sequence of the mouse-derived G protein coupled receptor protein cDNA fragment included in p3H2-17, wherein reverse base residues are in agreement.

Figure 77 is the nucleotide sequence of the open reading frame and neighboring regions thereof of human-derived G protein coupled receptor protein cDNA included in phAH2-17 and the amino acid sequence encoded thereby.

Figure 78 is the hydrophobicity plotting profile of the protein encoded by the human-derived G protein coupled receptor protein cDNA included in phAH2-17.

Figure 79 is the amino acid sequence of human type purinoceptor encoded by phAH2-17, relative to the mouse purinoceptor encoded by p3H2-17, wherein reverse amino acid residues are in agreement.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

According to the present invention, DNA sequences comprising each a nucleotide sequence indicated by a SEQ ID NO selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 19 have been synthesized and characterized. The DNA is a potent primer for polymerase chain reaction in order to amplify DNA sequences encoding part or all of the polypeptide sequence of G protein coupled receptor protein. PCR amplification methods of the DNA coding for part or all of the polypeptide sequence of G protein coupled receptor protein can be advantageously carried out with the said primer DNA. Screening of DNA libraries for the DNA encoding part or all of the polypeptide sequence of G protein coupled receptor protein can be successfully carried out through polymerase chain reaction techniques with the said primer DNA. As a result, template DNAs coding for part or all of the polypeptide sequence of G protein coupled receptor protein, contained in the DNA library, can be selectively amplified and various DNA sequences encoding part or all of the

polypeptide sequence of G protein coupled receptor protein may be isolated and characterized. Further, G protein coupled receptor proteins, peptide segments or fragments derived from the G protein coupled receptor protein, modified derivatives or analogues thereof, and salts thereof may be recognized, predicted, deduced, produced, expressed, isolated and characterized.

The primer DNA useful in PCR amplification of the DNA sequence encoding part or all of the polypeptide sequence of G protein coupled receptor protein is a degenerate deoxynucleotide which has an oligonucleotide sequence to which a SEQ ID NO selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 19 is assigned.

The nucleotide sequence represented by SEQ ID NO: 1 is a base sequence having the following formula:

5'-CGTGGSCMTSSTGGGCAACN₁YCCTG-3'

wherein S is G or C, M is A or C, N₁ = A, G, C, or T, and Y is T or C (Figure 1: HS-1).

The nucleotide sequence represented by SEQ ID NO: 2 (HS-2) is a base sequence having the following formula:

5'-GTN₁GWRRGGCAN₁CCAGCAGAKGGCAAA-3'

wherein N₁ = A, G, C, or T, W is A or T, R is A or G, and K is G or T, which is complementary to a nucleotide sequence having the following formula:

5'-TTTGCCMTCTGCTGGNTGCCYYWCNAC-3'

wherein N = A, C, G, or T, M is A or C, Y is T or C, and W is A or T (Figure 2).

The nucleotide sequence represented by SEQ ID NO: 3 is a base sequence having the following formula:

5'-CTCGCSGCMYTN₂RGYATGGAYCGN₂TAT-3'

wherein S is G or C, Y is C or T, M is A or C, R is A or G, and N₂ = I (Figure 4: 3D).

The nucleotide sequence represented by SEQ ID NO: 4 is a base sequence having the following formula:

5'-CATGTRGWAGGGAAN₂CCAGSAMAN₂RARRAA-3'

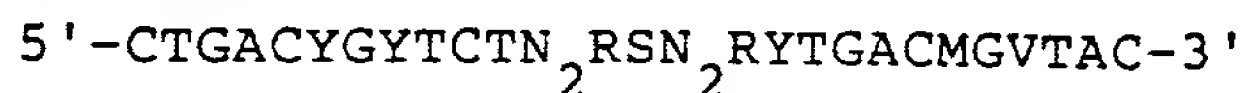
wherein R is A or G, W is T or A, S is G or C, M is A or C, and N₂ = I, which is complementary to a nucleotide sequence

having the following formula:



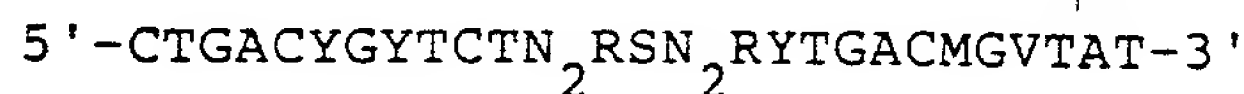
wherein Y is C or T, $N_1 = A, G, C, \text{ or } T$, K is G or T, S is G or C, W is A or T (Figure 6: 6C).

5 The nucleotide sequence represented by SEQ ID NO: 5 is a base sequence having the following formula:



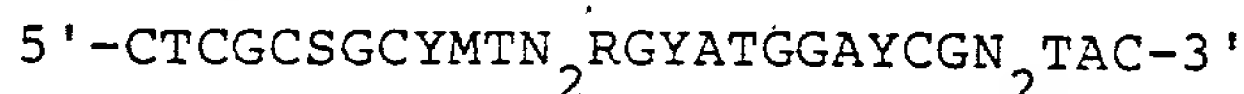
wherein Y is C or T, R is A or G, S is G or C, M is A or C, and V is A, C or G, and N_2 is I (Figure 3: 3A).

10 The nucleotide sequence represented by SEQ ID NO: 6 is a base sequence having the following formula:



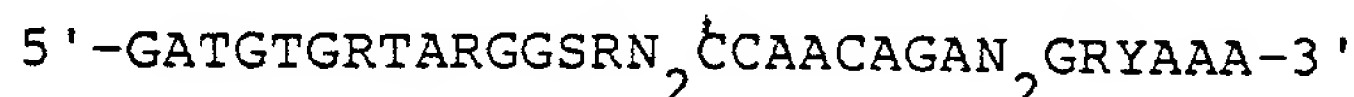
wherein Y is C or T, R is A or G, S is G or C, M is A or C, and V is A, C or G, and N_2 is I (Figure 3: 3B).

15 The nucleotide sequence represented by SEQ ID NO: 7 is a base sequence having the following formula:

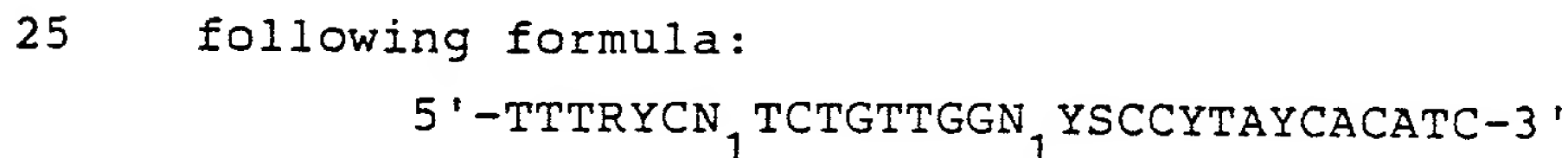


wherein S is G or C, Y is C or T, M is A or C, R is A or G, and N_2 is I (Figure 4: 3C).

20 The nucleotide sequence represented by SEQ ID NO: 8 is a base sequence having the following formula:

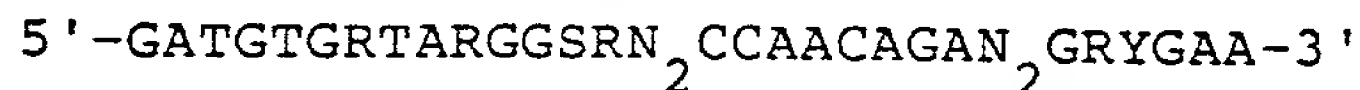


wherein R is A or G, S is G or C, Y is C or T, and N_2 is I, which is complementary to a nucleotide sequence having the following formula:

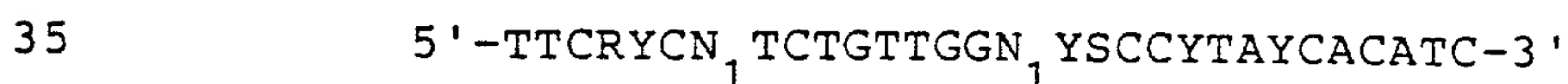


wherein R is A or G, Y is C or T, S is G or C, and N_1 is A, T, G, or C (Figure 5: 6A).

30 The nucleotide sequence represented by SEQ ID NO: 9 is a base sequence having the following formula:



wherein R is A or G, S is G or C, Y is C or T, and N_2 is I, which is complementary to a nucleotide sequence having the following formula:



wherein R is A or G, Y is C or T, S is G or C, and N_1 is A,

T, G, or C (Figure 5: 6B).

The nucleotide sequence represented by SEQ ID NO: 10 is a base sequence having the following formula:

5'-GYCACCAACN₂WSTTCATCCTSWN₂HCTG-3'

5 wherein S is G or C, Y is C or T, W is A or T, H is A, C or T, and N₂ is I (Figure 7: T2A).

The nucleotide sequence represented by SEQ ID NO: 11 (Figure 8: T7A) is a base sequence having the following formula:

10 5'-ASN₂SAN₂RAAGSARTAGAN₂GAN₂RGGRIT-3'

wherein R is A or G, S is G or C, and N₂ is I, which is complementary to a nucleotide sequence having the following formula:

5'-AAYCCYN₂TCN₂TCTAYTSCCTYN₂TSN₂ST-3'

15 wherein Y is C or T, N₂ is I, and S is G or C (Figure 8).

The nucleotide sequence represented by SEQ ID NO: 12 is a base sequence having the following formula:

5'-TGN₂TSSTKMTN₂GSN₂GTKGTN₂GGN₂AA-3'

20 wherein S is G or C, K is G or T, M is A or C, and N₂ is I (Figure 9: TM1-A2).

The nucleotide sequence represented by SEQ ID NO: 13 (Figure 10: TM3-B2) is a base sequence having the following formula:

5'-AYCKGTAYCKGTCCAN₂KGWN₂ATKGC-3'

25 wherein Y is C or T, K is G or T, W is A or T, and N₂ is I, which is complementary to a nucleotide sequence having the following formula:

5'-GCMATN₂WCMN₂TGGACMGRTACMGRT-3'

30 wherein M is A or C, W is A or T, R is A or G, and N₂ is I (Figure 10).

The nucleotide sequence represented by SEQ ID NO: 14 is a base sequence having the following formula:

5'-CATKKCCSTGGASAGN₂TAYN₂TRGC-3'

35 wherein K is G or T, S is G or C, Y is C or T, R is A or G, and N₂ is I (Figure 11: TM3-C2).

The nucleotide sequence represented by SEQ ID NO: 15 (Figure 12: TM6-E2) is a base sequence having the following

formula:

5'-GWWGGGSAKCCAGCASAN₂GGCRAA-3'

wherein W is A or T, S is G or C, K is G or T, R is A or G, and N₂ is I, which is complementary to a nucleotide sequence

5 having the following formula:

5'-TTYGCCN₂TSTGCTGGMTSCCCWWC-3'

wherein Y is C or T, S is G or C, M is A or C, W is A or T, and N₂ is I (Figure 12).

10 The nucleotide sequence represented by SEQ ID NO: 16 is a base sequence having the following formula:

5'-ARYYTN₂GCN₂N₂TN₂GCN₁GAY-3'

wherein R is A or G, Y is C or T, N₁ is A, T, G, or C, and N₂ is I (Figure 13: TM2F18).

15 The nucleotide sequence represented by SEQ ID NO: 17 (Figure 14: TM6R21) is a base sequence having the following formula:

5'-N₂GGN₂AN₂CCARCAN₁AN₁NRN₁RAA-3'

wherein R is A or G, N₁ is A, T, G, or C, and N₂ is I which is complementary to a nucleotide sequence having the following formula:

5'-TTYN₁YN₁N₁TN₁TGYTGGN₂TN₂CCN₂-3'

wherein Y is C or T, N₁ is A, T, G, or C, and N₂ is I (Figure 14).

25 The nucleotide sequence represented by SEQ ID NO: 18 is a base sequence having the following formula:

5'-GCCTSN₂TN₂RN₂SATGWSTGTGGAN₂MGN₂T-3'

wherein S is G or C, R is A or G, W is A or T, M is A or C, and N₂ is I (Figure 15: S3A).

30 The nucleotide sequence represented by SEQ ID NO: 19 (Figure 16: S6A) is a base sequence having the following formula:

5'-GAWSN₂TGMYN₂AN₂RTGGWAGGGN₂AN₂CCA-3'

wherein W is A or T, S is G or C, M is A or C, Y is C or T, R is A or G, and N₂ is I, which is complementary to a

35 nucleotide sequence having the following formula:

5'-TGGN₂TN₂CCCTWCCAYN₂TN₂RKCAN₂SWTC-3'

wherein W is A or T, Y is C or T, R is A or G, K is G or T, and S is G or C (Figure 16).

In a specific embodiment, symbols in the
aforementioned SEQ ID NOs (R, Y, M, K, S, W, H, V and N)
5 indicate the incorporation of plural bases, leading to multiple
oligonucleotides in the primer preparation. In other words,
SEQ ID NO: 1 to SEQ ID NO: 19 are degenerate nucleotide primers.

The nucleotide sequence represented by SEQ ID NO: 1
(Figure 1: HS-1) is a nucleotide sequence highly homologous to
10 the DNA sequence coding for the amino acid sequence
corresponding to or near the first membrane-spanning
(transmembrane) domain each of known G protein coupled receptor
proteins such as human-derived TRH receptor protein (HTRHR),
human-derived RANTES receptor protein (L10918, HUMRANTES),
15 human Burkitt's lymphoma-derived receptor protein with an
unknown ligand (X68149, HSBLR1A), human-derived
somatostatin receptor protein (L14856, HUMSOMAT0), rat-derived
 μ -opioid receptor protein (U02083, RNU02083), rat-derived
 κ -opioid receptor protein (U00442, U00442), human-derived
20 neuromedin B receptor protein (M73482, HUMNMBR),
human-derived muscarinic acetylcholine receptor
protein (X15266, HSHM4), rat-derived adrenaline
 α_1 B receptor protein (L08609, RATAADRE01), human-derived
somatostatin 3 receptor protein (M96738, HUMSSTR3X),
25 human-derived C_5a receptor protein (HUMC5AAR), human-derived
receptor protein with an unknown ligand (HUMRDC1A),
human-derived receptor protein with an unknown ligand
(M84605, HUMOPIODRE), rat-derived adrenaline
 α_2 B receptor protein (M91466, RATA2BAR) and the like
30 [Figure 1].

The nucleotide sequence represented by SEQ ID NO: 2
(HS-2) is a nucleotide sequence which is complementary to the
nucleotide sequence (Figure 2) highly homologous to the DNA
sequence coding for the amino acid sequence corresponding to or
35 near the sixth membrane-spanning domain of known G protein
coupled receptor proteins such as mouse-derived receptor

protein with an unknown ligand (M80481, MUSGIR), human-derived bombesin receptor protein (L08893, HUMBOMB3S), human-derived adenosine A2 receptor protein (S46950, S46950), mouse-derived receptor protein with an unknown ligand (D21061, MUSGPCR),
5 mouse-derived TRH receptor protein (S43387, S43387),
rat-derived neuromedin K receptor protein (J05189, RATNEURA),
rat-derived adenosine A1 receptor protein (M69045, RATA1ARA),
human-derived neurokinin A receptor protein (M57414, HUMNEKAR),
rat-derived adenosine A3 receptor protein (M94152, RATADENREC),
10 human-derived somatostatin 1 receptor protein (M81829, HUMSTRI1A), human-derived neurokinin 3 receptor protein (S86390, S86371S4), rat-derived receptor protein with an unknown ligand (X61496, RNCGPCR), human-derived somatostatin 4 receptor protein (L07061, HUMSSTR4Z), rat-derived GnRH
15 receptor protein (M31670, RATGNRHA) and the like [Figure 2].

The nucleotide sequence represented by SEQ ID NO: 5 (Figure 3: 3A) or the nucleotide sequence represented by SEQ ID NO: 6 (Figure 3: 3B) is a nucleotide sequence highly homologous to the DNA sequence coding for the amino acid
20 sequence corresponding to or near the third membrane-spanning domain each of known G protein coupled receptors such as mouse-derived κ -opioid receptor protein (L11064), mouse-derived δ -opioid receptor protein (L11065), rat-derived μ -opioid receptor protein (D16349), mouse-derived bradykinin
25 B2 receptor protein (X69676), rat-derived bradykinin B2 receptor protein (M59967), mouse-derived bombesin receptor protein (M35328), human-derived neuromedin B receptor protein (M73482), human-derived gastrin releasing peptide receptor protein (M73481), human-derived bombesin receptor protein
30 subtype 3 (L08893), mouse-derived substance K receptor protein (X62933), mouse-derived substance P receptor protein (X62934), rat-derived neurokinin 3 receptor protein (J05189), rat-derived endothelin receptor protein (M60786), rat-derived receptor protein with an unknown ligand (L04672), rat-derived
35 receptor protein with an unknown ligand (X61496), rat-derived receptor protein with an unknown ligand (X59249), rat-derived receptor protein with an unknown ligand (L09249),

mouse-derived receptor protein with an unknown ligand (P30731), human-derived receptor protein with an unknown ligand (M31210), human-derived receptor protein with an unknown ligand (U03642) and the like [Figure 3].

5 The nucleotide sequence represented by SEQ ID NO: 7 (Figure 4: 3C) or the nucleotide sequence represented by SEQ ID NO: 3 (Figure 4: 3D) is a nucleotide sequence highly homologous to the DNA sequence coding for the amino acid
10 sequence corresponding to or near the third membrane-spanning domain each of known G protein coupled receptors such as mouse-derived angiotensin II receptor protein (L32840), rat-derived angiotensin Ib receptor protein (X64052), rat-derived angiotensin receptor protein subtype (M90065), human-derived angiotensin Ia receptor protein (M91464),
15 rat-derived cholecystokinin a receptor protein (M88096), rat-derived cholecystokinin b receptor protein (M99418), human-derived cholecystokinin b receptor protein (L04473), mouse-derived low affinity interleukin 8 receptor protein (M73969), human-derived high affinity interleukin 8 receptor
20 protein (X65858), mouse-derived C5a anaphylatoxin receptor protein (S46665), human-derived N-formylpeptide receptor protein (M60626) and the like [Figure 4].

 The nucleotide sequence represented by SEQ ID NO: 10 (Figure 7: T2A) is a nucleotide sequence highly homologous to
25 the DNA sequence coding for the amino acid sequence corresponding to or near the second membrane-spanning domain each of known G protein coupled receptors such as human galanin receptor (HUMGALAREC), rat α -1B-adrenergic receptor (RATADR1B), human β -1-adrenergic receptor
30 (HUMADRB1), rabbit IL-8 receptor (RABIL8RSB), human opioid receptor (HUMOPIODRE), bovine substance K receptor (BTSKR), human somatostatin receptor-2 (HUMSRI2A), human somatostatin receptor-3 (HUMSSTR3Y), human gastrin receptor (HUMGARE), human cholecystokinin A receptor (HUMCCKAR), human dopamine
35 receptor-D5 (HUMD1B), human serotonin receptor 5HT1E (HUM5HT1E), human dopamine receptor D4 (HUMD4C), mouse serotonin receptor-2 (MMSERO), rat α -1A-adrenergic receptor

(RATADRA1A), rat histamine H2 receptor (S57565) and the like [Figure 7].

5 The nucleotide sequence represented by SEQ ID NO: 8 (complementary to 6A of Figure 5) or the nucleotide sequence represented by SEQ ID NO: 9 (complementary to 6B of Figure 5) is a nucleotide sequence which is complementary to the nucleotide sequence (Figure 5) highly homologous to the DNA sequence coding for the amino acid sequence corresponding to or near the sixth membrane-spanning domain of known G protein coupled receptors such as mouse-derived κ -opioid receptor protein (L11064), mouse-derived δ -opioid receptor protein (L11065), rat-derived μ -opioid receptor protein (D16349), mouse-derived bradykinin B2 receptor protein (X69676), rat-derived bradykinin B2 receptor protein (M59967), 10 mouse-derived bombesin receptor protein (M35328), human-derived neuromedin B receptor protein (M73482), human-derived gastrin releasing peptide receptor protein (M73481), human-derived bombesin receptor protein subtype 3 (L08893), mouse-derived substance K receptor protein (X62933), 15 mouse-derived substance P receptor protein (X62934), rat-derived neurokinin 3 receptor protein (J05189), rat-derived endothelin receptor protein (M60786), rat-derived receptor protein with an unknown ligand (L04672), rat-derived receptor protein with an unknown ligand (X61496), 20 rat-derived receptor protein with an unknown ligand (X59249), rat-derived receptor protein with an unknown ligand (L09249), mouse-derived receptor protein with an unknown ligand (P30731), human-derived receptor protein with an unknown ligand (M31210) human-derived receptor protein with an unknown ligand (U03642) 25 and the like [Figure 5]. 30

The nucleotide sequence represented by SEQ ID NO: 4 (complementary to 6C of Figure 6) is a nucleotide sequence which is complementary to the nucleotide sequence (Figure 6) highly homologous to the DNA sequence coding for the amino acid sequence corresponding to or near the sixth membrane-spanning domain of known G protein coupled receptors such as 35 mouse-derived angiotensin II receptor protein (L32840),

rat-derived angiotensin Ib receptor protein (X64052),
rat-derived angiotensin receptor protein subtype (M90065),
human-derived angiotensin Ia receptor protein (M91464),
rat-derived cholecystokinin a receptor protein (M88096),
5 rat-derived cholecystokinin b receptor protein (M99418),
human-derived cholecystokinin 8 receptor protein (L04473),
mouse-derived low affinity interleukin 8 receptor protein
(M73969), human-derived high affinity interleukin 8 receptor
protein (X65858), mouse-derived C5a anaphylatoxin receptor
10 protein (S46665), human-derived N-formylpeptide receptor
protein (M60626) and the like [Figure, 6].

The nucleotide sequence represented by SEQ ID NO: 11
(Figure 8: T7A) is a nucleotide sequence which is
complementary to the nucleotide sequence (Figure 8) highly
15 homologous to the DNA sequence coding for the amino acid
sequence corresponding to or near the seventh
membrane-spanning domain each of known G protein coupled
receptors such as human galanin receptor (HUMGALAREC),
rat A1 adenosine receptor (RAT1DREC), porcine angiotensin
20 receptor (PIGA2R), rat serotonin receptor (RAT5HTRTC),
human dopamine receptor (S58541), human gastrin releasing
peptide receptor (HUMGRPR), mouse GRP/bombesin receptor
(MUSGRPBOM), rat vascular type 1 angiotensin receptor
(RRVT1AIIR), human muscarinic acetylcholine receptor (HSHM4),
25 human β -1 adrenergic receptor (HUMDRB1), human gastrin
receptor (HUMGARE), rat cholecystokinin receptor (RATCCKAR),
rat receptor with an unknown ligand (S59748), human
somatostatin receptor (HUMSST28A), rat receptor with an unknown
ligand (RNGPROCR), mouse somatostatin receptor-1 (MUSSRI1A),
30 human α -A1-adrenergic receptor (HUMA1AADR), mouse
delta-opioid receptor (S66181), human somatostatin receptor-3
(HUMSSTR3Y) and the like [Figure 8].

The nucleotide sequence represented by SEQ ID NO: 12
(Figure 9: TM1-A2) is a nucleotide sequence highly homologous
35 to the DNA sequence coding for the amino acid sequence
within the first membrane-spanning (transmembrane) domain
each of known G protein coupled receptors such as

mouse-derived bradykinin B₂ receptor (MUSBB2R),
bovine-derived substance K receptor (BTSKR), bovine-derived
endothelin ET_B receptor (BOVEETBR), human-derived
neuropeptide Y receptor (MMSUBKREC), human-derived
5 prostaglandin E₂ receptor (HUMPGE2R), human-derived
prostacyclin receptor (HUMPIR), human-derived κ -opioid
receptor (HSU11053), rat-derived melanocortin 3 receptor
(RRMC3RA), human-derived melanocortin receptor (HUMMR),
mouse-derived bombesin/GRP receptor (MUSGRPBOM),
10 rat-derived cholecystokinin B receptor (RATCHOLREC),
rat-derived cholecystokinin A receptor (RATCCKAR) and the
like [Figure 9].

The nucleotide sequence represented by SEQ ID NO: 13
(Figure 10: TM3-B2) is a nucleotide sequence which
15 is complementary to the nucleotide sequence (Figure 10) highly
homologous to the DNA sequence coding for the amino acid
sequence corresponding to or near the end of the third
membrane-spanning domain of known G protein coupled receptors
such as human-derived cholecystokinin receptor (HUMCCKR),
20 human-derived cholecystokinin B receptor (HUMCCKBGR),
mouse-derived melanocortin 5 receptor (MMGMC5R),
human-derived vasopressin receptor (HUMV2R), rat-derived
neuromedin K receptor (RATNEURA), dog-derived gastrin receptor
(DOGGSTRN), rat-derived serotonin receptor (RAT5HT5A),
25 mouse-derived α_2 -adrenaline receptor (MUSALP2ADA),
human-derived adenosine A₁ receptor (HUMADORA1X),
human-derived opioid (presumed) receptor (HUMOPIODRE),
mouse-derived bombesin/GRP receptor (MUSGRPBOM),
rat-derived cholecystokinin A receptor (RATCCKAR),
30 human-derived TRH receptor (HSTRHREC) and the like [Figure 10].

The nucleotide sequence represented by SEQ ID NO: 14
(Figure 11: TM3-C2) is a nucleotide sequence highly homologous
to the DNA sequence coding for the amino acid sequence
corresponding to or near the end of the third membrane-spanning
35 domain of known G protein coupled receptors such as
human-derived neurokinin 3 receptor (HUMNK3R), human-derived
oxytocin receptor (HSMRNAOXY), guinea pig-derived

cholecystokinin A receptor (S68242), dog-derived
cholecystokinin A receptor with an unknown ligand (CFGPCR4),
mouse-derived substance P receptor (MMSUBPREC), human-derived
receptor with an unknown ligand (HUMOPIODRE), human-derived
5 galanin receptor (HUMGALAREC), human-derived serotonin
receptor (HSS31G), human-derived β_3 -adrenaline receptor
(HUMARB3A), human-derived prostacyclin receptor (HUMHPR),
rat-derived cholecystokinin A receptor (RATCCKAR) and the
like [Figure 11].

10 The nucleotide sequence represented by SEQ ID NO: 15
(Figure 12: TM6-E2) is a nucleotide sequence which
is complementary to the nucleotide sequence (Figure 12) highly
homologous to the DNA sequence coding for the amino acid
sequence within the sixth membrane-spanning domain of known
15 G protein coupled receptors such as human-derived neurokinin A
receptor (HUMNEKAR), human-derived substance P receptor
(HUMSUBPRA), rat-derived substance K receptor (RATSKR),
mouse-derived bombesin/GRP receptor (MUSGRPBOM),
human-derived opioid (presumed) receptor (HUMOPIODRE),
20 human-derived adenosine A₂ receptor (HUMA2XXX),
human-derived β_2 -adrenaline receptor (HUMADRBR),
canine-derived receptor RDC5 with an unknown ligand (CFGPCR8),
human-derived endothelin receptor (HUMETSR), mouse-derived
neuropeptide Y1 receptor (MMNPY1CDS), human-derived oxytocin
25 receptor (HSMRNOXY), rat-derived cholecystokinin A receptor
(RATCCKAR) and the like [Figure 12].

The nucleotide sequence represented by SEQ ID NO: 16
(Figure 13: TM2F18) is a nucleotide sequence highly homologous
to the DNA sequence coding for the amino acid sequence
30 corresponding to or near the second membrane-spanning domain
of known G protein coupled receptors such as human-derived TSH
receptor (HUMTSHX), human-derived neurokinin A receptor
(HUMNEKAR), human-derived FMLP receptor (HUMFMLP),
human-derived IL8 receptor B (HUMINTLEU8), human-derived
35 α -A1 adrenergic receptor (HUMA1AADR), human-derived IL8
receptor A (HUMIL8RA), human-derived dopamine D2 receptor
(HSDD2), human-derived angiotensin type I receptor (HUMANTIR),

human-derived somatostatin receptor (HUSOMAT), human-derived TRH receptor (HSTRHREC), human-derived delta-opioid receptor (HSUO7882) and the like [Figure 13].

The nucleotide sequence represented by SEQ ID NO: 17 (Figure 14: TM6R21) is a nucleotide sequence which is complementary to the nucleotide sequence (Figure 14) highly homologous to the DNA sequence coding for the amino acid sequence corresponding to or near the sixth membrane-spanning domain of known G protein coupled receptors such as human-derived β -adrenergic receptor (HSBAR), human-derived neurokinin A receptor (HUMNEKAR), human-derived endothelin-1 receptor (HUMETN1R), human-derived histamine H_2 receptor (HUMHISH2R), human-derived α -A1 adrenergic receptor (HUMA1AADR), human-derived IL8 receptor A (HUMIL8RA), human-derived neuromedin B receptor (HUMNMBR), human-derived neurokinin 1 receptor (HUMNKIRX), human-derived substance P receptor (HUMSUBPRA), human-derived 5-HT1D serotonin receptor (HUM5HT1DA), human-derived formylpeptide receptor (HUMPFPR2A), human-derived dopamine D2 receptor (HSDD2), human-derived neuropeptide Y receptor (HUMNEUYREC), human-derived adenosine A2 receptor (HUMA2XXX), human-derived bradykinin receptor BK-2 (HUMBK2A), human-derived FMLP-related receptor II (HUMFMLPX), human-derived somatostatin receptor subtype 3 (HUMSSTR3X), human-derived cholecystokinin receptor (HUMCCKR), human-derived neurotensin receptor (HSNEURA) and the like [Figure 14].

The nucleotide sequence represented by SEQ ID NO: 18 (Figure 15: S3A) is a nucleotide sequence highly homologous to the DNA sequence coding for the amino acid sequence corresponding to or near the third membrane-spanning domain of known G protein coupled receptors such as human-derived galanin receptor (HUMGALAREC), human-derived CCK-B receptor (S70057), human-derived ET_A receptor (S67127), human-derived ET_B receptor (S44866), human-derived C5A receptor (HUMC5AAR), human-derived angiotensin II receptor (HUMANTIR), human-derived bradykinin receptor (HUMBK2R), human-derived neurotensin receptor (HSNEURA), human-derived GRP receptor (HUMGRPR), human-derived somatostatin 5 receptor (HUMFSRS),

human-derived IL-8 receptor (HUMIL8RA), human-derived neurokinin 2 (neurokinin A) receptor (HUMNEKAR) and the like [Figure 15].

The nucleotide sequence represented by SEQ ID NO: 19 (Figure 16: S6A) is a nucleotide sequence which is complementary to the nucleotide sequence (Figure 16) highly homologous to the DNA sequence coding for the amino acid sequence corresponding to or near the sixth membrane-spanning domain of known G protein coupled receptors such as human-derived galanin receptor (HUMGLAREC), human-derived CCK-B receptor (S70057), human-derived ET_A receptor (S67127), human-derived ET_B receptor (S44866), human-derived C5A receptor (HUMC5AAR), human-derived angiotensin II receptor (HUMANTIR), human-derived bradykinin receptor (HUMBK2R), human-derived neurotensin receptor (HSNEURA), human-derived GRP receptor (HUMGRPR), human-derived somatostatin 5 receptor (HUMFSRS), human-derived IL-8 receptor (HUMIL8RA), human-derived neurokinin 2 (neurokinin A) receptor (HUMNEKAR) and the like [Figure 16].

The above-mentioned abbreviations in the parentheses are the identifiers (or reference numbers) which are shown when GenBank/EMBL Data Bank is searched using a DNASIS Gene/Protein Sequence Data Base (CD019; Hitachi Software Engineering, Japan) and are usually called "Accession Numbers" or "Entry Names". HTRHR is, however, the sequence as described in Japanese Patent Application No. Hei 5-286986 (or No. 286986/1993) (EPA 638645).

The DNA (or nucleotides) of the present invention may be manufactured by DNA synthetic methods which are known per se or by methods similar thereto. The DNA (or nucleotides) of the present invention may be an oligonucleotide sequence having 8 to 60 base residues, preferably 12 to 50 base residues, more preferably 15 to 40 residues and most preferably 18 to 30 residues.

Among the DNAs of the present invention, the DNA having the nucleotide sequence represented by SEQ ID NO: 1 or

SEQ ID NO: 12 is a nucleotide sequence which is commonly present in the nucleotide sequence of the DNA encoding the amino acid sequence corresponding to or near the first membrane-spanning domain of the above-mentioned known G protein coupled receptor protein. Therefore, it can be complementarily bonded (i.e. is hybridizable) with RNA or DNA (including genome DNA, cDNA) coding for the amino acid sequence corresponding to or near the first membrane-spanning domain of known or unknown G protein coupled receptor proteins and, furthermore, it can be complementarily bonded (i.e. is hybridizable) with nucleotide sequences encoding other membrane-spanning domains as well.

The DNA having a nucleotide sequence represented by SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 14 or SEQ ID NO: 18 is a nucleotide sequence which is commonly present in the nucleotide sequence of the DNA coding for the amino acid sequence corresponding to or near the third membrane-spanning domain of the above-mentioned known G protein coupled receptor protein. Therefore, it can be complementarily bonded with RNA or DNA (including genome DNA, cDNA) coding for the part corresponding to or near the third membrane-spanning domain of known or unknown G protein coupled receptor proteins and, furthermore, it can be complementarily bonded with nucleotide sequences encoding other membrane-spanning domains as well.

The DNA having a nucleotide sequence represented by SEQ ID NO: 10 or SEQ ID NO: 16 is a nucleotide sequence which is commonly present in the nucleotide sequence of the DNA coding for the amino acid sequence corresponding to or near the second membrane-spanning domain of the above-mentioned known G protein coupled receptor protein. Therefore, it can be complementarily bonded with RNA or DNA (including genome DNA, cDNA) coding for the part corresponding to or near the second membrane-spanning domain of known or unknown G protein coupled receptor proteins and, furthermore, it can be complementarily bonded with nucleotide sequences encoding other membrane-spanning domains as well.

The DNA having a nucleotide sequence represented by
SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID
NO: 15, SEQ ID NO: 17 or SEQ ID NO: 19 is a nucleotide sequence
which is commonly present in the nucleotide sequence of the DNA
coding for the amino acid sequence corresponding to or near
the sixth membrane-spanning domain of the above-mentioned known
G protein coupled receptor protein. Therefore, it can be
complementarily bonded with RNA or DNA (including genome DNA,
cDNA) coding for the part corresponding to or near the sixth
membrane-spanning domain of known or unknown G protein coupled
receptor proteins and, furthermore, it can be complementarily
bonded with nucleotide sequences encoding other
membrane-spanning domains as well.

The DNA having a nucleotide sequence represented by
SEQ ID NO: 11 is a nucleotide sequence which is commonly
present in the nucleotide sequence of the DNA coding for the
amino acid sequence corresponding to or near the seventh
membrane-spanning domain of the above-mentioned known G protein
coupled receptor protein. Therefore, it can be complementarily
bonded with RNA or DNA (including genome DNA, cDNA) coding for
the part corresponding to or near the seventh membrane-spanning
domain of known or unknown G protein coupled receptor proteins
and, further more, it can be complementarily bonded with
nucleotide sequences encoding other transmembrane domains
as well.

The DNA having a nucleotide sequence represented by
SEQ ID NO: 13 is a nucleotide sequence which is commonly
present in the nucleotide sequence of the DNA coding for the
amino acid sequence corresponding to or near the third
membrane-spanning domain of the above-mentioned known G protein
coupled receptor protein. Therefore, it can be complementarily
bonded with RNA or DNA (including genome DNA, cDNA) coding for
the part corresponding to or near the third membrane-spanning
domain of known or unknown G protein coupled receptor proteins
and, furthermore, it can be complementarily bonded with
nucleotide sequences encoding other membrane-spanning domains
as well.

Accordingly, the DNAs (or nucleotides) of the present invention can be used as DNA primers for a polymerase chain reaction (hereinafter, sometimes referred to as PCR).

For example:

- 5 (i) a polymerase chain reaction is carried out by mixing

(1) a small amount of DNA (or DNA fragment(s)) which codes for G protein coupled receptor protein, said DNA (or DNA fragment(s)) acting as a template,

- 10 (2) at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 1, DNA primers having a nucleotide sequence represented by SEQ ID NO: 3, DNA primers having a nucleotide sequence represented by SEQ ID NO: 5, DNA primers having a nucleotide sequence represented by SEQ ID NO: 6, DNA primers having a nucleotide sequence represented by SEQ ID NO: 7, DNA primers having a nucleotide sequence represented by SEQ ID NO: 10, DNA primers having a nucleotide sequence represented by SEQ ID NO: 12, DNA primers having a nucleotide sequence represented by SEQ ID NO: 14, DNA primers having a nucleotide sequence represented by SEQ ID NO: 16 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 18 and

- 25 (3) at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 2, DNA primers having a nucleotide sequence represented by SEQ ID NO: 4, DNA primers having a nucleotide sequence represented by SEQ ID NO: 8, DNA primers having a nucleotide sequence represented by SEQ ID NO: 9, DNA primers having a nucleotide sequence represented by SEQ ID NO: 11, DNA primers having a nucleotide sequence represented by SEQ ID NO: 15, DNA primers having a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 19; or
- 35

- (ii) a polymerase chain reaction is carried out by mixing

(1) a small amount of DNA (or DNA fragment(s)) coding for G protein coupled receptor protein, said DNA (or DNA fragment(s)) acting as a template,

(2) at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 1 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 12 and

(3) at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 13

so that it is possible to amplify the target DNA (or DNA fragment(s)) coding for said receptor protein.

When the PCR is carried out using at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 2, DNA primers having a nucleotide sequence represented by SEQ ID NO: 4, DNA primers having a nucleotide sequence represented by SEQ ID NO: 8, DNA primers having a nucleotide sequence represented by SEQ ID NO: 9, DNA primers having a nucleotide sequence represented by SEQ ID NO: 11, DNA primers having a nucleotide sequence represented by SEQ ID NO: 15, DNA primers having a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 19, said DNA primer(s) is(are) bonded (hybridized) with the nucleotide sequence at the 3'-side of the + chain (plus chain) of template RNA or DNA (or fragment(s) thereof) coding for the sixth membrane-spanning domain or other membrane-spanning domains of G protein coupled receptor protein whereupon an elongation of the - chain (minus chain) proceeds in the 5' → 3' direction.

When the PCR is carried out using at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 11, said DNA primer is bonded with the nucleotide sequence at the 3'-side of the + chain (plus chain) of template RNA or DNA (or fragment(s) thereof) coding for the seventh membrane-spanning domain or other membrane-spanning domains of the G protein

coupled receptor protein whereupon an elongation of the - chain (minus chain) proceeds in the 5' → 3' direction.

When the PCR is carried out using at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 1 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 12, said DNA primer is bonded with the nucleotide sequence at the 3'-side of the - chain (minus chain) of template RNA or DNA (or fragment(s) thereof) coding for the first membrane-spanning domain or other membrane-spanning domains of G protein coupled receptor protein whereupon an elongation of the + chain (plus chain) proceeds in the 5' → 3' direction.

When the PCR is carried out using at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 10 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 16, said DNA primer is bonded with the nucleotide sequence at the 3'-side of the - chain (minus chain) of template RNA or DNA (or fragment(s) thereof) coding for the second membrane-spanning domain or other membrane-spanning domains of G protein coupled receptor protein whereupon an elongation of the + chain (plus chain) proceeds in the 5' → 3' direction.

When the PCR is carried out using at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 3, DNA primers having a nucleotide sequence represented by SEQ ID NO: 5, DNA primers having a nucleotide sequence represented by SEQ ID NO: 6, DNA primers having a nucleotide sequence represented by SEQ ID NO: 7, DNA primers having a nucleotide sequence represented by SEQ ID NO: 14 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 18, said DNA primer is bonded with the nucleotide sequence at the 3'-side of the - chain (minus chain) of template RNA or DNA (or fragment(s) thereof) coding for the third membrane-spanning domain or other membrane-spanning domains of G protein coupled receptor protein whereupon an elongation of the + chain (plus chain) proceeds in the 5' → 3' direction.

Accordingly, when the DNA primers having nucleotide sequences represented by any of SEQ ID NO: 1 to SEQ ID NO: 19 of the present invention are used in combination each other, DNA (or DNA fragment(s)) coding for G protein coupled receptor protein can be successfully amplified.

One embodiment of the present invention provides:

(A) a method of amplifying DNA coding for the G protein coupled receptor protein (e.g., from the first to sixth membrane-spanning (transmembrane) domains or other segments of the G protein coupled receptor protein), characterized in that a polymerase chain reaction is carried out by mixing

① a DNA coding for the G protein coupled receptor protein, said DNA acting as a template,

② at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 1 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 12 and

③ at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 2, DNA primers having a nucleotide sequence represented by SEQ ID NO: 4, DNA primers having a nucleotide sequence represented by SEQ ID NO: 8, DNA primers having a nucleotide sequence represented by SEQ ID NO: 9, DNA primers having a nucleotide sequence represented by SEQ ID NO: 15, DNA primers having a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 19;

(B) a method of amplifying DNA coding for the G protein coupled receptor protein (e.g., from the first to seventh membrane-spanning (transmembrane) domains or other segments of the G protein coupled receptor protein), characterized in that a polymerase chain reaction is carried out by mixing

① a DNA coding for the G protein coupled receptor protein, said DNA acting as a template,

② at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 1 and DNA primers having a

nucleotide sequence represented by SEQ ID NO: 12 and

③ at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO:11;

5 (C) a method of amplifying a DNA coding for the G protein coupled receptor protein (e.g., from the second to sixth membrane-spanning (transmembrane) domains or other segments of the G protein coupled receptor protein), characterized in that a polymerase chain reaction is carried out by mixing

10 ① a DNA coding for the G protein coupled receptor protein, said DNA acting as a template,

② at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 10 and DNA primers having a
15 nucleotide sequence represented by SEQ ID NO: 16 and

③ at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 2, DNA primers having a nucleotide sequence represented by SEQ ID NO: 4, DNA primers having a
20 nucleotide sequence represented by SEQ ID NO: 8, DNA primers having a nucleotide sequence represented by SEQ ID NO: 9, DNA primers having a nucleotide sequence represented by SEQ ID NO: 15, DNA primers having a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers having a
25 nucleotide sequence represented by SEQ ID NO: 19;

(D) a method of amplifying a DNA coding for the G protein coupled receptor protein (e.g., from the second to seventh membrane-spanning (transmembrane) domains or other segments of the G protein coupled receptor protein), characterized in
30 that a polymerase chain reaction is carried out by mixing

① a DNA coding for the G protein coupled receptor protein, said DNA acting as a template,

② at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 10 and DNA primers having a
35 nucleotide sequence represented by SEQ ID NO: 16 and

③ at least one DNA primer selected from the group

consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 11;

(E) a method of amplifying a DNA coding for the G protein coupled receptor protein (e.g., from the third to sixth membrane-spanning (transmembrane) domains or other segments of the G protein coupled receptor protein), characterized in that a polymerase chain reaction is carried out by mixing

① a DNA coding for the G protein coupled receptor protein, said DNA acting as a template,

② at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 3, DNA primers having a nucleotide sequence represented by SEE ID NO: 5, DNA primers having a nucleotide sequence represented by SEQ ID NO: 6, DNA primers having a nucleotide sequence represented by SEQ ID NO: 7, DNA primers having a nucleotide sequence represented by SEQ ID NO: 14 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 18 and

③ at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 2, DNA primers having a nucleotide sequence represented by SEQ ID NO: 4, DNA primers having a nucleotide sequence represented by SEQ ID NO: 8, DNA primers having a nucleotide sequence represented by SEQ ID NO: 9, DNA primers having a nucleotide sequence represented by SEQ ID NO: 15, DNA primers having a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 19;

(F) a method of amplifying a DNA coding for the G protein coupled receptor protein (e.g., from the third to seventh membrane-spanning (transmembrane) domains or other segments of the G protein coupled receptor protein), characterized in that a polymerase chain reaction is carried out by mixing

① a DNA coding for the G protein coupled receptor protein, said DNA acting as a template,

② at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence

represented by SEQ ID NO: 3, DNA primers having a nucleotide sequence represented by SEQ ID NO: 5, DNA primers having a nucleotide sequence represented by SEQ ID NO: 6, DNA primers having a nucleotide sequence represented by SEQ ID NO: 7,
5 DNA primers having a nucleotide sequence represented by SEQ ID NO: 14 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 18 and

③ at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence
10 represented by SEQ ID NO:11; and

(G) a method of amplifying a DNA coding for the G protein coupled receptor protein (e.g., from the first to third membrane-spanning (transmembrane) domains or other segments of the G protein coupled receptor protein), characterized in
15 that a polymerase chain reaction is carried out by mixing

① a DNA coding for the G protein coupled receptor protein, said DNA acting as a template,

② at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence
20 represented by SEQ ID NO: 1 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 12 and

③ at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 13.

25 An example of more preferred combination of the DNA primers in the amplification according to the above-mentioned (A) includes a combination of a DNA primer having a nucleotide sequence represented by SEQ ID NO: 1 with a DNA primer having a nucleotide sequence represented by SEQ ID NO: 2 and the
30 like.

An example of more preferred combination of the DNA primers in the amplification according to the above-mentioned (D) includes a combination of a DNA primer having a nucleotide sequence represented by SEQ ID NO: 10 with a DNA primer having
35 a nucleotide sequence represented by SEQ ID NO: 11 and the like.

An example of more preferred combination of the DNA

primers in the amplification according to the above-mentioned (E) includes:

(i) a combination of a DNA primer having a nucleotide sequence represented by SEQ ID NO: 5 or a DNA primer having a nucleotide sequence represented by SEQ ID NO: 6 with a DNA primer having a nucleotide sequence represented by SEQ ID NO: 8 or a DNA primer having a nucleotide sequence represented by SEQ ID NO: 9;

(ii) a combination of a DNA primer having a nucleotide sequence represented by SEQ ID NO: 3 or a DNA primer having a nucleotide sequence represented by SEQ ID NO: 7 with a DNA primer having a nucleotide sequence represented by SEQ ID NO: 4 and the like.

An example of more preferred combination of the DNA primers in the amplification according to the above-mentioned (G) includes a combination of a DNA primer having a nucleotide sequence represented by SEQ ID NO: 12 with a DNA primer having a nucleotide sequence represented by SEQ ID NO: 13 and the like.

The amplification may be carried out in accordance with known PCR techniques. For example, it may be carried out by the method described in Saiki, R. K. et al., Science, 239:487-491 (1988). Temperature, time, buffer, number of reaction cycles, enzyme such as DNA polymerase, addition of 2'-deoxy-7-deazaguanosine triphosphate or inosine, etc. in the PCR amplification may be suitably selected depending upon the type of target DNA and other factors.

When RNA is used as a template, PCR amplification may be carried out, for example, by the method described in Saiki, R. K. et al., Science, 239:487-491(1988).

Moreover, the DNA having a nucleotide sequence represented by SEQ ID NO: 1 or SEQ ID NO: 12 of the present invention can be selectively and complementarily bonded (hybridized) with the nucleotide sequence at the 3'-side of the - chain of the DNA coding for the amino acid sequence corresponding to or near the first membrane-spanning domain of the G protein coupled receptor protein; the DNA having a nucleotide sequence represented by SEQ ID NO: 10 or SEQ ID

NO: 16 of the present invention can be selectively and complementarily bonded (hybridized) with the nucleotide sequence at the 3'-side of the - chain of the DNA coding for the amino acid sequence corresponding to or near the second
5 membrane-spanning domain of the G protein coupled receptor protein; the DNA having a nucleotide sequence represented by SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 14 or SEQ ID NO: 18 of the present invention can be selectively and complementarily bonded (hybridized) with the
10 nucleotide sequence at the 3'-side of the - chain of the DNA coding for the amino acid sequence corresponding to or near the third membrane-spanning domain of the G protein coupled receptor protein; the DNA having a nucleotide sequence represented by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 8,
15 SEQ ID NO: 9, SEQ ID NO: 15, SEQ ID NO: 17 or SEQ ID NO: 19 of the present invention can be selectively and complementarily bonded (hybridized) with the nucleotide sequence at the 3'-side of the + chain of the DNA coding for the amino acid sequence corresponding to or near the sixth membrane-spanning domain
20 of the G protein coupled receptor protein; the DNA having a nucleotide sequence represented by SEQ ID NO: 11 of the present invention can be selectively and complementarily bonded (hybridized) with the nucleotide sequence at the 3'-side of the + chain of the DNA coding for the amino acid
25 sequence corresponding to or near the third membrane-spanning domain of the G protein coupled receptor protein; and the DNA having a nucleotide sequence represented by SEQ ID NO: 13 of the present invention can be selectively and complementarily bonded (hybridized) with the nucleotide sequence at the
30 3'-side of the + chain of the DNA coding for the amino acid sequence corresponding to or near the third membrane-spanning domain of the G protein coupled receptor protein and, accordingly, said DNA is also advantageously useful as a
35 probe for screening DNA libraries for DNA (or DNA fragment(s)) encoding part or all of the polypeptide sequence of G protein coupled receptor proteins.

These screening methods for DNA (or DNA fragment(s))

encoding part or all of the polypeptide sequence of G protein coupled receptor proteins from the DNA library by using as a reagent, because it can be used as a probe the DNA of the present invention may be carried out according to DNA cloning methods known per se by those of skill in the art or methods similar thereto. Especially when the DNA of the present invention is used as a DNA primer for the PCR, both amplification and screening of the DNA (or DNA fragment) coding for the G protein coupled receptor protein can be conducted in a single step.

Thus, when the DNAs of the present invention are suitably combined and used as the DNA primer for the PCR, said DNA primer(s) is(are) bonded (hybridized) with RNA or DNA (or fragment(s) thereof) encoding the amino acid sequence of the first membrane-spanning (transmembrane) domain, the second membrane-spanning domain, the third membrane-spanning domain, the sixth membrane-spanning domain, the seventh membrane-spanning domain or other membrane-spanning domains of G protein coupled receptor proteins to amplify, for example,

- ① RNA or DNA (or fragment(s) thereof) coding for the amino acid sequence of from the first membrane-spanning to the sixth membrane-spanning domains of G protein coupled receptor proteins,
- ② RNA or DNA (or fragment(s) thereof) coding for the amino acid sequence of from the first membrane-spanning to the seventh membrane-spanning domains of G protein coupled receptor proteins,
- ③ RNA or DNA (or fragment(s) thereof) coding for the amino acid sequence of from the third membrane-spanning to the sixth membrane-spanning domains of G protein coupled receptor proteins,
- ④ RNA or DNA (or fragment(s) thereof) coding for the amino acid sequence of from the third membrane-spanning to the seventh membrane-spanning domains of G protein coupled receptor proteins,
- ⑤ RNA or DNA (or fragment(s) thereof) coding for the amino acid sequence of from the second membrane-spanning to the

sixth membrane-spanning domains of G protein coupled receptor proteins or RNA or DNA (or fragment(s) thereof) coding for the amino acid sequence of other domains thereof,

5 ⑥ RNA or DNA (or fragment(s) thereof) coding for the amino acid sequence of from the second membrane-spanning to the seventh membrane-spanning domains of G protein coupled receptor proteins,

10 ⑦ RNA or DNA (or fragment(s) thereof) coding for the amino acid sequence of from the first membrane-spanning to the third membrane-spanning domains of G protein coupled receptor proteins or

⑧ RNA or DNA (or fragment(s) thereof) coding for the amino acid sequence of other domains of G protein coupled receptor proteins.

15 Through using the DNA primer according to the present invention, therefore, selective amplifications of:

20 ① RNA or DNA (or fragment(s) thereof) coding for the amino acid sequence covering from the first membrane-spanning domain to the sixth membrane-spanning domain of G protein coupled receptor proteins;

② RNA or DNA (or fragment(s) thereof) coding for the amino acid sequence covering from the first membrane-spanning domain to the seventh membrane-spanning domain of G protein coupled receptor proteins;

25 ③ RNA or DNA (or fragment(s) thereof) coding for the amino acid sequence covering from the third membrane-spanning domain to the sixth membrane-spanning domain of G protein coupled receptor proteins;

30 ④ RNA or DNA (or fragment(s) thereof) coding for the amino acid sequence covering from the third membrane-spanning domain to the seventh membrane-spanning domain of G protein coupled receptor proteins;

35 ⑤ RNA or DNA (or fragment(s) thereof) coding for the amino acid sequence covering from the second membrane-spanning domain to the sixth membrane-spanning domain of G protein coupled receptor proteins or RNA or DNA (or fragment(s) thereof) coding for the amino acid sequence covering other areas thereof,

⑥ RNA or DNA (or fragment(s) thereof) coding for the amino acid sequence covering from the second membrane-spanning domain to the seventh membrane-spanning domain of G protein coupled receptor proteins;

5 ⑦ RNA or DNA (or fragment(s) thereof) coding for the amino acid sequence covering from the first membrane-spanning domain to the third membrane-spanning domain of G protein coupled receptor proteins; and the like, from DNA libraries can be successfully achieved.

10 Among the DNA primers of the present invention, the combination of

① a DNA primer having a nucleotide sequence represented by SEQ ID NO: 1 or SEQ ID NO: 2; with

15 ② at least one DNA primer selected from the group consisting of a DNA primer having a nucleotide sequence represented by SEQ ID NO: 2, a DNA primer having a nucleotide sequence represented by SEQ ID NO: 4, a DNA primer having a nucleotide sequence represented by SEQ ID NO: 8, a DNA primer having a nucleotide sequence represented by SEQ ID NO: 9, a DNA primer having a nucleotide sequence represented by SEQ ID NO: 15, a DNA primer having a nucleotide sequence represented by SEQ ID NO: 17 and a DNA primer having a nucleotide sequence represented by SEQ ID NO: 19;

20 is, unlike conventional primers, capable of selectively amplifying a broad area covering from the first membrane-spanning domain to the sixth membrane-spanning domain or other domains of G protein coupled receptor proteins.

Among the DNA primers of the present invention, the combination of

30 ① a DNA primer having a nucleotide sequence represented by SEQ ID NO: 1 or SEQ ID NO: 12; with

② a DNA primer having a nucleotide sequence represented by SEQ ID NO: 11;

35 is, unlike conventional primers, capable of selectively amplifying a broad area covering from the first membrane-spanning domain to the seventh membrane-spanning domain or other domains of G protein coupled receptor proteins.

Among the DNA primers of the present invention, the combination of

① a DNA primer having a nucleotide sequence represented by SEQ ID NO: 10 or SEQ ID NO: 16; with

5 ② at least one DNA primer selected from the group consisting of a DNA primer having a nucleotide sequence represented by SEQ ID NO: 2, a DNA primer having a nucleotide sequence represented by SEQ ID NO: 4, a DNA primer having a nucleotide sequence represented by SEQ ID NO: 8, a DNA primer having a nucleotide
10 sequence represented by SEQ ID NO: 9, a DNA primer having a nucleotide sequence represented by SEQ ID NO: 15, a DNA primer having a nucleotide sequence represented by SEQ ID NO: 17 and a DNA primer having a nucleotide sequence represented by SEQ ID NO: 19;

15 is, unlike conventional primers, capable of selectively amplifying a broad area covering from the second membrane-spanning domain to the sixth membrane-spanning domain or other domains of G protein coupled receptor proteins.

Among the DNA primers of the present invention, the combination of

① a DNA primer having a nucleotide sequence represented by SEQ ID NO:10 or SEQ ID NO:16; with

② a DNA primer having a nucleotide sequence represented by SEQ ID NO:11;

25 is, unlike conventional primers, capable of selectively amplifying a broad area covering from the second membrane-spanning domain to the seventh membrane-spanning domain or other domains of G protein coupled receptor proteins.

Among the DNA primers of the present invention, the combination of

① at least one DNA primer selected from the group consisting of a DNA primer having a nucleotide sequence represented by SEQ ID NO: 3, a DNA primer having a nucleotide sequence represented by SEQ ID NO: 5, a DNA primer having a nucleotide sequence
35 represented by SEQ ID NO: 6, a DNA primer having a nucleotide sequence represented by SEQ ID NO: 7, a DNA primer having a nucleotide sequence represented by SEQ ID NO: 14 and a DNA

primer having a nucleotide sequence represented by SEQ ID NO: 18; with

② a DNA primer having a nucleotide sequence represented by SEQ ID NO: 11;

5 is, unlike conventional primers, capable of selectively amplifying a broad area covering from the third membrane-spanning domain to the seventh membrane-spanning domain or other domains of G protein coupled receptor proteins.

Therefore, the protein hydrophobicity plotting of G
10 protein coupled receptor proteins and the homology at the amino acid level or the nucleic acid level between G protein coupled receptor proteins and other similar receptor proteins [said hydrophobicity plotting and homology both serve as standards
15 thereof) obtained according to the present invention is(are) encoding part or all of the amino acid sequence of G protein coupled receptor protein] can now be more clearly calculated.

Among the DNA primers of the present invention, the combination of

20 ① at least one DNA primer selected from the group consisting of a DNA primer having a nucleotide sequence represented by SEQ ID NO: 3, a DNA primer having a nucleotide sequence represented by SEQ ID NO: 5, a DNA primer having a nucleotide sequence represented by SEQ ID NO: 6, a DNA primer having a nucleotide
25 sequence represented by SEQ ID NO: 7, a DNA primer having a nucleotide sequence represented by SEQ ID NO: 14 and a DNA primer having a nucleotide sequence represented by SEQ ID NO: 18; with

30 ② at least one DNA primer selected from the group consisting of a DNA primer having a nucleotide sequence represented by SEQ ID NO: 2, a DNA primer having a nucleotide sequence represented by SEQ ID NO: 4, a DNA primer having a nucleotide sequence represented by SEQ ID NO: 8, a DNA primer having a nucleotide sequence represented by SEQ ID NO: 9, a DNA primer having a
35 nucleotide sequence represented by SEQ ID NO: 15, a DNA primer having a nucleotide sequence represented by SEQ IS NO: 17 and a DNA primer having a nucleotide sequence represented by SEQ ID

NO: 19;

is capable of amplifying the areas covering from the third membrane-spanning domain to the sixth membrane-spanning domain thereof at once like the conventional DNA primers and,
5 moreover, it is capable of more selectively and efficiently amplifying DNA coding for G protein coupled receptor proteins though it has not been obtained through the conventional DNA primers.

Moreover, among the DNA primers of the present
10 invention, the combination of

① at least one DNA primer selected from DNA primers having a nucleotide sequence of SEQ ID NO: 1 and DNA primers having a nucleotide sequence of SEQ ID NO: 12; with

② a DNA primer having a nucleotide sequence represented by
15 SEQ ID NO: 13;

is capable of amplifying the areas covering from the first membrane-spanning domain to the third membrane-spanning domain thereof at once.

Then (a) the amplified DNA (or fragment(s) thereof)
20 coding for the amino acid sequence of from the first membrane-spanning domain to the sixth membrane-spanning domain of G protein coupled receptor protein, (b) the amplified DNA (or fragment(s) thereof) coding for the amino acid sequence of from the first membrane-spanning domain to the seventh
25 membrane-spanning domain of G protein coupled receptor protein, (c) the amplified DNA (or fragment(s) thereof) coding for the amino acid sequence of from the third membrane-spanning domain to the sixth membrane-spanning domain of G protein coupled receptor protein, (d) the amplified DNA (or fragment(s)
30 thereof) coding for the amino acid sequence of from the third membrane-spanning domain to the seventh membrane-spanning domain of G protein coupled receptor protein, (e) the amplified DNA (or fragment(s) thereof) coding for the amino acid sequence of from the second membrane-spanning domain to the sixth
35 membrane-spanning domain of G protein coupled receptor protein, (f) the amplified DNA (or fragment(s) thereof) coding for the amino acid sequence of from the second membrane-spanning domain

to the seventh membrane-spanning domain of G protein coupled receptor protein, (g) the amplified DNA (or fragment(s) thereof) coding for the amino acid sequence of from the first membrane-spanning domain to the third membrane-spanning domain of G protein coupled receptor protein or (h) the amplified DNA (or fragment(s) thereof) coding for the amino acid sequence of other domains of G protein coupled receptor protein may be used as a probe(s) to screen for full-length DNA which completely encodes G protein coupled receptor proteins from DNA libraries according to methods known per se by those of skill in the art or methods similar thereto.

The DNA libraries used in the present invention include any of genome DNA libraries, cDNA libraries and RNA libraries. The term "DNA library" or "DNA libraries" as used herein refers to a DNA library or DNA libraries including all of those libraries.

The present invention further provides screening methods for target DNA (or fragment(s) thereof) coding for G protein coupled receptor protein from the DNA library containing DNA (or fragment(s) thereof) coding for receptor proteins, which comprise employing the DNA of the present invention as a DNA primer for the PCR.

One preferred embodiment of the present invention is a method for cloning full-length DNA which completely encodes an amino acid sequence of G protein coupled receptor protein from DNA libraries which comprises the steps of

- (i) using the DNA of the present invention as a DNA primer for PCR;
- (ii) carrying out PCR in the presence of a mixture of said DNA primer with the DNA library to amplify and select (i.e. screen for) a DNA fragment coding for the amino acid sequence of from the first membrane-spanning domain to the sixth membrane-spanning domain of G protein coupled receptor protein, a DNA fragment coding for the amino acid sequence of from the first membrane-spanning domain to the seventh membrane-spanning domain of G protein coupled receptor protein, a DNA fragment coding for the amino acid sequence of from the third membrane-

spanning domain to the sixth membrane-spanning domain of G protein coupled receptor protein, a DNA fragment coding for the amino acid sequence of from the third membrane-spanning domain to the seventh membrane-spanning domain of G protein coupled receptor protein, a DNA fragment coding for the amino acid sequence of from the second membrane-spanning domain to the sixth membrane-spanning domain of G protein coupled receptor protein, a DNA fragment coding for the amino acid sequence of from the second membrane-spanning domain to the seventh membrane-spanning domain of G protein coupled receptor protein, a DNA fragment coding for the amino acid sequence of from the first membrane-spanning domain to the third membrane-spanning domain of G protein coupled receptor protein or a DNA fragment coding for other domains of G protein coupled receptor protein; and

(iii) cloning said full-length DNA from the DNA library according to cloning methods known per se by those of skill in the art or methods similar thereto by using, as a probe, the DNA fragment obtained in the above step (ii).

Preferably, an embodiment of the present invention is a screening method of DNA coding for G protein coupled receptor proteins from DNA libraries, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

- ① the DNA library,
- ② at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 1, DNA primers having a nucleotide sequence represented by SEQ ID NO: 3, DNA primers having a nucleotide sequence represented by SEQ ID NO: 5, DNA primers having a nucleotide sequence represented by SEQ ID NO: 6, DNA primers having a nucleotide sequence represented by SEQ ID NO: 7, DNA primers having a nucleotide sequence represented by SEQ ID NO: 10, DNA primers having a nucleotide sequence represented by SEQ ID NO: 14, DNA primers having a nucleotide sequence represented by SEQ ID NO: 16 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 18 and

③ at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 2, DNA primers having a nucleotide sequence represented by SEQ ID NO: 4, DNA primers having a nucleotide sequence represented by SEQ ID NO: 8, DNA primers having a nucleotide sequence represented by SEQ ID NO: 9, DNA primers having a nucleotide sequence represented by SEQ ID NO: 11, DNA primers having a nucleotide sequence represented by SEQ ID NO: 15, DNA primers having a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 19 to selectively amplify template DNA coding for G protein coupled receptor protein contained in the DNA library.

More preferably, embodiments of the present invention include:

(1) a screening method of DNA coding for the amino acid sequence of G protein coupled receptor protein and the like (e.g. the regions spanning from the first transmembrane domain to the sixth transmembrane domain of G protein coupled receptor protein or other domains thereof) from a DNA library, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

① the DNA library,

② at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 1 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 12 and

③ at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 2, DNA primers having a nucleotide sequence represented by SEQ ID NO: 4, DNA primers having a nucleotide sequence represented by SEQ ID NO: 8, DNA primers having a nucleotide sequence represented by SEQ ID NO: 9, DNA primers having a nucleotide sequence represented by SEQ ID NO: 15, DNA primers having a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 19

to selectively amplify the DNA coding for the amino acid sequence of G protein coupled receptor protein and the like (e.g. the regions spanning from the first transmembrane domain to the sixth transmembrane domain of G protein coupled receptor protein or other domains thereof) contained in the DNA library;

(2) a screening method of DNA coding for the amino acid sequence of G protein coupled receptor protein and the like (e.g. the regions spanning from the first transmembrane domain to the seventh transmembrane domain of G protein coupled receptor protein or other domains thereof) from a DNA library, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

① the DNA library,

② at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 1 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 12 and

③ at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 11

to selectively amplify the DNA coding for the amino acid sequence of G protein coupled receptor protein and the like (e.g. the regions spanning from the first transmembrane domain to the seventh transmembrane domain of G protein coupled receptor protein or other domains thereof) contained in the DNA library;

(3) a screening method of DNA coding for the amino acid sequence of G protein coupled receptor protein and the like (e.g. the regions spanning from the second transmembrane domain to the sixth transmembrane domain of G protein coupled receptor protein or other domains thereof) from a DNA library, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

① the DNA library,

② at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by

SEQ ID NO: 10 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 16 and

③ at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 2, DNA primers having a nucleotide sequence represented by SEQ ID NO: 4, DNA primers having a nucleotide sequence represented by SEQ ID NO: 8, DNA primers having a nucleotide sequence represented by SEQ ID NO: 9, DNA primers having a nucleotide sequence represented by SEQ ID NO: 15, DNA primers having a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 19

to selectively amplify the DNA coding for the amino acid sequence of G protein coupled receptor protein and the like (e.g. the regions spanning from the second transmembrane domain to the sixth transmembrane domain of G protein coupled receptor protein or other domains thereof) contained in the DNA library;

(4) a screening method of DNA coding for the amino acid sequence of G protein coupled receptor protein and the like (e.g. the regions spanning from the second transmembrane domain to the seventh transmembrane domain of G protein coupled receptor protein or other domains thereof) from a DNA library, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

① the DNA library,

② at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 10 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 16 and

③ at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 11

to selectively amplify the DNA coding for the amino acid sequence of G protein coupled receptor protein and the like (e.g. the regions spanning from the second transmembrane domain to the seventh transmembrane domain of G protein coupled

receptor protein or other domains thereof) contained in the DNA library;

(5) a screening method of DNA coding for the amino acid sequence of G protein coupled receptor protein and the like (e.g. the regions spanning from the third transmembrane domain to the sixth transmembrane domain of G protein coupled receptor protein or other domains thereof) from a DNA library, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

① the DNA library,

② at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 3, DNA primers having a nucleotide sequence represented by SEQ ID NO: 5, DNA primers having a nucleotide sequence represented by SEQ ID NO: 6, DNA primers having a nucleotide sequence represented by SEQ ID NO: 7, DNA primers having a nucleotide sequence represented by SEQ ID NO: 14 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 18 and

③ at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 2, DNA primers having a nucleotide sequence represented by SEQ ID NO: 4, DNA primers having a nucleotide sequence represented by SEQ ID NO: 8, DNA primers having a nucleotide sequence represented by SEQ ID NO: 9, DNA primers having a nucleotide sequence represented by SEQ ID NO: 15, DNA primers having a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 19

to selectively amplify the DNA coding for the amino acid sequence of G protein coupled receptor protein and the like (e.g. the regions spanning from the third transmembrane domain to the sixth transmembrane domain of G protein coupled receptor protein or other domains thereof) contained in the DNA library;

(6) a screening method of DNA coding for the amino acid sequence of G protein coupled receptor protein and the like

(e.g. the regions spanning from the third transmembrane domain to the seventh transmembrane domain of G protein coupled receptor protein or other domains thereof) from a DNA library, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

① the DNA library,

② at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 3, DNA primers having a nucleotide sequence

represented by SEQ ID NO: 5, DNA primers having a nucleotide sequence represented by SEQ ID NO: 6, DNA primers having a nucleotide sequence represented by SEQ ID NO: 7, DNA primers having a nucleotide sequence represented by SEQ ID NO: 14 and DNA primers having a nucleotide sequence represented by

SEQ ID NO: 18 and

③ at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 11

to selectively amplify the DNA coding for the amino acid sequence of G protein coupled receptor protein and the like (e.g. the regions spanning from the third transmembrane domain to the seventh transmembrane domain of G protein coupled receptor protein or other domains thereof) contained in the DNA library; and

(7) a screening method of DNA coding for the amino acid sequence of G protein coupled receptor protein and the like (e.g. the regions spanning from the first transmembrane domain to the third transmembrane domain of G protein coupled receptor protein or other domains thereof) from a DNA library, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

① the DNA library,

② at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by

SEQ ID NO: 1 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 12 and

③ at least one DNA primer selected from the group consisting

of DNA primers having a nucleotide sequence represented by
SEQ ID NO: 13

to selectively amplify the DNA coding for the amino acid
sequence of G protein coupled receptor protein and the like
5 (e.g. the regions spanning from the first transmembrane
domain to the third transmembrane domain of G protein coupled
receptor protein or other domains thereof) contained in
the DNA library.

Particularly preferably, embodiments of the present
10 invention include:

(8) a screening method of DNA coding for the amino acid
sequence of G protein coupled receptor protein from a DNA
library, which comprises carrying out a polymerase chain
reaction in the presence of a mixture of

- 15 ① the DNA library,
② a DNA primer having a nucleotide sequence represented by
SEQ ID NO: 1 and
③ a DNA primer having a nucleotide sequence represented by
SEQ ID NO: 2

20 to selectively amplify the DNA coding for the amino acid
sequence of G protein coupled receptor protein contained in
the DNA library;

(9) a screening method of DNA coding for the amino acid
sequence of G protein coupled receptor protein from a DNA
25 library, which comprises carrying out a polymerase chain
reaction in the presence of a mixture of

- ① the DNA library,
② a DNA primer having a nucleotide sequence represented by
SEQ ID NO: 3 and
30 ③ a DNA primer having a nucleotide sequence represented by
SEQ ID NO: 4

to selectively amplify the DNA coding for the amino acid
sequence of G protein coupled receptor protein contained in
the DNA library;

35 (10) a screening method of DNA coding for the amino acid
sequence of G protein coupled receptor protein from a DNA
library, which comprises carrying out a polymerase chain

reaction in the presence of a mixture of

① the DNA library,

② a DNA primer having a nucleotide sequence represented by
SEQ ID NO: 6 and

5 ③ a DNA primer having a nucleotide sequence represented by
SEQ ID NO: 8

to selectively amplify the DNA coding for the amino acid
sequence of G protein coupled receptor protein contained in
the DNA library; and

10 (11) a screening method of DNA coding for the amino acid
sequence of G protein coupled receptor protein from a DNA
library, which comprises carrying out a polymerase chain
reaction in the presence of a mixture of

① the DNA library,

15 ② a DNA primer having a nucleotide sequence represented by
SEQ ID NO: 10 and

③ a DNA primer having a nucleotide sequence represented by
SEQ ID NO: 11

20 to selectively amplify the DNA coding for the amino acid
sequence of G protein coupled receptor protein contained in
the DNA library.

The cloned DNAs can be analyzed, usually by
restriction enzyme analysis and/or sequencing.

25 Target RNA or DNA (or fragment(s) thereof) coding
for G protein coupled receptor protein in the amplification
and the screening by the PCR techniques wherein the DNA of
the present invention is employed may include RNA, DNA or
fragments thereof coding for known (or prior art) G protein
coupled receptor proteins and RNA, DNA or fragments thereof
30 coding for unknown (novel) G protein coupled receptor
proteins.

These target RNA or DNA (or fragment(s) thereof) may
include novel nucleotide sequences and even known nucleotide
sequences.

35 Examples of such nucleotide sequences are RNA or DNA
(or fragment(s)) coding for a G protein coupled receptor
protein, said RNA or DNA (or fragment(s)) being derived from

all cells and tissues (e.g. pituitary gland, brain, pancreas, lung, adrenal gland, etc.) of vertebrate animals (e.g. mice, rats, cats, dogs, swines, cattle, horses, monkeys, human beings, etc.), insects or other invertebrate animals (e.g. drosophilae, silkworms, Barathra brassicae, etc.), plants (e.g. rice plant, wheat, tomato, etc.) and cultured cell lines derived therefrom, etc.

Specific examples of the nucleotide sequences are RNA or DNA (or fragment(s)) coding for G protein coupled receptor proteins such as receptor proteins to angiotensin, bombesin, canavaninoid, cholecystokinin, glutamine, serotonin, melatonin, neuropeptide Y, opioid, purine, vasopressin, oxytocin, VIP (vasoactive intestinal and related peptide), somatostatin, dopamine, motilin, amylin, bradykinin, CGRP (calcitonin gene related peptide), adrenomedullin, leukotriene, pancreastatin, prostaglandin, thromboxane, adenosine, adrenaline, α - and β -chemokine (IL-8, GRO α , GRO β , GRO γ , NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, MIP1 α , MIP-1 β , RANTES, etc.), endothelin, enterogastrin, histamine, neurotensin, TRH, pancreatic polypeptide, galanin, family members thereof, etc.

In the PCR amplification using the DNA of the present invention, the DNA (or DNA fragment) acting as a template may include any DNA so far as it is derived from the above-mentioned tissues and cells. More specifically, the template DNA (or DNA fragment) includes any of genome DNA, genome DNA libraries, cDNA derived from the tissues and cells and cDNA libraries derived from the tissues and cells. cDNA libraries derived from human tissues and cells are particularly suitable. Vectors to be used in the DNA library may include any of bacteriophages, plasmids, cosmids, phagimids, etc. It is also possible to directly amplify the template DNA (or DNA fragment) by reverse transcriptase polymerase chain reaction (RT-PCR) techniques using mRNA fractions prepared from the tissues and cells. The DNA which is to be a template may be either DNA completely coding for G protein coupled receptor proteins or DNA fragments (or

segments) thereof.

Preferably, the RNA or DNA (or fragment(s) thereof) obtained via the instant screening method for G protein coupled receptor protein coding DNA wherein said method uses the DNA according to the present invention is a G protein coupled receptor protein-encoding RNA or DNA (or fragment(s) thereof) contained in the used DNA library. More specifically, it is an RNA or DNA (or RNA fragment(s) or DNA fragment(s) (hereinafter, may be often abbreviated as just "DNA") coding for G protein coupled receptor proteins such as angiotensin receptor, bombesin receptor, canavaninoid receptor, cholecystokinin receptor, glutamine receptor, serotonin receptor, melatonin receptor, neuropeptide Y receptor, opioid receptor, purine receptor, vasopressin receptor, oxytocin receptor, VIP receptor (vasoactive intestinal and related peptide receptor), somatostatin receptor, dopamine receptor, motilin receptor, amylin receptor, bradykinin receptor, CGRP receptor (calcitonin gene related peptide receptor), adrenomedullin receptor, leukotriene receptor, pancreastatin receptor, prostaglandin receptor, thromboxane receptor, adenosine receptor, adrenaline receptor, α - and β -chemokine receptor (receptors to IL-8, GRO α , GRO β , GRO γ , NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, MIP1 α , MIP-1 β , RANTES, etc.), endothelin receptor, enterogastrin receptor, histamine receptor, neurotensin receptor, TRH receptor, pancreatic polypeptide receptor, galanin receptor, their family member receptors, etc.

When the DNA obtained by the screening method of the present invention is the DNA fragment which partially codes for a G protein coupled receptor protein, it is possible to isolate DNA completely encoding said G protein coupled receptor protein from a suitable DNA library according to cloning techniques known per se by using said DNA fragment as a probe.

Means for cloning the DNA completely encoding G protein coupled receptor proteins may include a PCR amplification employing a synthetic DNA primer having the partial nucleotide sequence of the DNA fragment partially

coding for the G protein coupled receptor protein and a selection of the target DNA via a hybridization with DNA or synthetic DNA having part or all of the region of said DNA fragments. The hybridization may be conducted, for example, by the methods described in Molecular Cloning, 2nd ed.; J. Sambrook et al., Cold Spring Harbor Lab. Press, 1989. When the commercially available library is used, it may be conducted according to the manners described in the protocols attached thereto.

The DNA completely encoding G protein coupled receptor protein (full-length G protein coupled receptor protein DNA) may be used, depending upon its object, either as it is or after digesting with a restriction enzyme or after ligating with a linker if desired. Said DNA may have ATG at the 5'-terminal as the translation initiation codon and TAA, TGA or TAG at the 3' terminal as the translation termination codon. These translation initiation codons and translation termination codons may be added using a suitable synthetic DNA adaptor. In addition, it is possible to determine said receptor protein-expressing tissues/cells by northern blottings using said DNA as a probe. It is also possible to express target receptor proteins by introducing DNA having the entire coding region of the receptor protein into animal cells after binding with a suitable promoter.

The G protein coupled receptor protein according to the present invention is a G protein coupled receptor protein encoded by the G protein coupled receptor protein-encoding DNA obtained by the screening method of the present invention. More specifically, the G protein coupled receptor protein according to the present invention includes G protein coupled receptor proteins such as angiotensin receptor protein, bombesin receptor protein, canavanoid receptor protein, cholecystokinin receptor protein, glutamine receptor protein, serotonin receptor protein, melatonin receptor protein, neuropeptide Y receptor protein, opioid receptor protein, purine receptor protein, vasopressin receptor protein, oxytocin receptor protein, VIP receptor protein (vasoactive

intestinal and related peptide receptor protein), somatostatin receptor protein, dopamine receptor protein, motilin receptor protein, amylin receptor protein, bradykinin receptor protein, CGRP receptor protein (calcitonin gene related peptide receptor protein), adrenomedullin receptor protein, leukotriene receptor protein, pancreastatin receptor protein, prostaglandin receptor protein, thromboxane receptor protein, adenosine receptor protein, adrenaline receptor protein, α - and β -chemokine receptor protein (receptor protein responsive to IL-8, GRO α , GRO β , GRO γ , NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, MIP1 α , MIP-1 β , RANTES, etc.), endothelin receptor protein, enterogastrin receptor protein, histamine receptor protein, neurotensin receptor protein, TRH receptor protein, pancreatic polypeptide receptor protein, galanin receptor protein, family members thereof, etc.

According to the present invention, novel G protein coupled receptors proteins, peptide segments or fragments derived from the G protein coupled receptor protein, modified derivatives or analogues thereof, and salts thereof may be recognized, cloned, produced, isolated or characterized.

These G protein coupled receptor proteins are those derived from all cells and tissues (e.g. pituitary gland, pancreas, brain, kidney, liver, gonad, thyroid gland, cholecyst, bone marrow, adrenal, skin, muscle, lung, digestive duct, blood vessel, heart, etc.) of warm-blooded animals (e.g. guinea pig, rat, mouse, swine, sheep, cattle, monkey, human beings, rabbit, cat, dog, horse, etc.), and any of proteins as long as they comprise an amino acid sequence selected from the group consisting of an amino acid sequence represented by SEQ ID NO: 24, an amino acid sequence represented by SEQ ID NO: 25, an amino acid sequence represented by SEQ ID NO: 26, an amino acid sequence represented by SEQ ID NO: 27, an amino acid sequence represented by SEQ ID NO: 28, an amino acid sequence represented by SEQ ID NO: 34, an amino acid sequence represented by SEQ ID NO: 35, an amino acid sequence represented by SEQ ID NO: 38, an amino acid sequence represented by SEQ ID NO: 39, an amino acid sequence

represented by SEQ ID NO: 56, and substantial equivalents to the amino acid sequence represented by SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 38, SEQ ID NO: 39, and/or SEQ ID NO: 56.

In one embodiment of the present invention, G protein coupled receptor proteins are those derived from all cells and tissues (e.g. pituitary gland, pancreas, brain, kidney, liver, gonad, thyroid gland, cholecyst, bone marrow, adrenal, skin, muscle, lung, digestive duct, blood vessel, heart, etc.) of warm-blooded animals (e.g. guinea pig, rat, mouse, swine, sheep, cattle, monkey, human beings, cat, dog, horse, etc.), and any of proteins as long as they comprise an amino acid sequence selected from the group consisting of an amino acid sequence represented by SEQ ID NO: 24, an amino acid sequence represented by SEQ ID NO: 25, an amino acid sequence represented by SEQ ID NO: 26, an amino acid sequence represented by SEQ ID NO: 27, an amino acid sequence represented by SEQ ID NO: 28, and substantial equivalents to the amino acid sequence represented by SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, or SEQ ID NO: 28. These G protein coupled receptor proteins may include proteins having an amino acid sequence selected from the group consisting of an amino acid sequence represented by SEQ ID NO: 24, an amino acid sequence represented by SEQ ID NO: 25, an amino acid sequence represented by SEQ ID NO: 26, an amino acid sequence represented by SEQ ID NO: 27 and an amino acid sequence represented by SEQ ID NO: 28, proteins wherein the amino acid sequence thereof is about 90% to 99.9% homologous to an amino acid sequence represented by SEQ ID NO: 24, an amino acid sequence represented by SEQ ID NO: 25, an amino acid sequence represented by SEQ ID NO: 26, an amino acid sequence represented by SEQ ID NO: 27 or an amino acid sequence represented by SEQ ID NO: 28 and the activity thereof is substantially equivalent to the protein having an amino acid sequence represented by SEQ ID NO: 24, an amino acid sequence represented by SEQ ID NO: 25, an amino acid sequence

represented by SEQ ID NO: 26, an amino acid sequence
represented by SEQ ID NO: 27 or an amino acid sequence
represented by SEQ ID NO: 28 and the like. The substantially
equivalent activity may include ligand binding activity,
5 signal information transmitting, etc. The term "substantially
equivalent" or "substantial equivalent" means that the nature
of the ligand binding activity and the like is equivalent.
Therefore, it is allowable that even differences among grades
such as ligand binding affinity grades and ligand binding
10 activity grades and quantitative factors such as molecular
weights of receptor proteins are present.

In another embodiment of the present invention, G
protein coupled receptor proteins include human pituitary
gland-derived G protein coupled receptor proteins comprising
15 an amino acid sequence selected from the group consisting of
an amino acid sequence represented by SEQ ID NO: 24, and/or
an amino acid sequence represented by SEQ ID NO: 25, mouse
pancreas-derived G protein coupled receptor proteins comprising
an amino acid sequence represented by SEQ ID NO: 27, mouse
20 pancreas-derived G protein coupled receptor proteins comprising
an amino acid sequence represented by SEQ ID NO: 28, etc.
Examples of the human pituitary gland-derived G protein coupled
receptor protein comprising an amino acid sequence selected
from the group consisting of an amino acid sequence represented
25 by SEQ ID NO: 24, and an amino acid sequence represented by SEQ
ID NO: 25, are human pituitary gland-derived G protein coupled
receptor proteins comprising an amino acid sequence represented
by SEQ ID NO: 24, etc. These G protein coupled receptor
proteins may include proteins wherein one or more amino acid
30 residues (preferably from 2 to 30 amino acid residues, more
preferably from 2 to 10 amino acid residues) are deleted from
the amino acid sequence of SEQ ID NO: 24, SEQ ID NO: 25, SEQ
ID NO: 26, SEQ ID NO: 27 or SEQ ID NO: 28, proteins wherein one
or more amino acid residues (preferably from 2 to 30 amino acid
35 residues, more preferably from 2 to 10 amino acid residues) are
added to the amino acid sequence of SEQ ID NO: 24, SEQ ID NO:
25, SEQ ID NO: 26, SEQ ID NO: 27 or SEQ ID NO: 28, proteins

wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) in the amino acid sequence of SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27 or SEQ ID NO: 28, are substituted with one or more amino acid residues, etc.

In yet another embodiment of the present invention, G protein coupled receptor proteins include those derived from all cells and tissues (e.g. amygdaloid nucleus, pituitary gland, pancreas, brain, kidney, liver, gonad, thyroid gland, cholecyst, bone marrow, lung, digestive duct, blood vessel, heart, thymus, spleen, leukocyte, etc.) of warm-blooded animals (e.g. guinea pig, rat, mouse, pig, sheep, cattle, monkey, human beings, etc.), and any of proteins as long as they comprise an amino acid sequence selected from the group consisting of an amino acid sequence represented by SEQ ID NO: 34 and/or an amino acid sequence represented by SEQ ID NO: 35. These G protein coupled receptor proteins may include proteins having an amino acid sequence selected from the group consisting of an amino acid sequence represented by SEQ ID NO: 34 or/and an amino acid sequence represented by SEQ ID NO: 35, proteins wherein the amino acid sequence thereof is about 90% to 99.9% homologous to an amino acid sequence represented by SEQ ID NO: 34 or/and an amino acid sequence represented by SEQ ID NO: 35 and the activity thereof is substantially equivalent to the protein having an amino acid sequence represented by SEQ ID NO: 34 and/or an amino acid sequence represented by SEQ ID NO: 35, and the like. The substantially equivalent activity may include ligand binding activity, signal information transmitting, etc. The term "substantially equivalent" or "substantial equivalent" means that the nature of the ligand binding activity and the like is equivalent. Therefore, it is allowable that even differences among grades such as ligand binding affinity grades and ligand binding activity grades and quantitative factors such as molecular weights of receptor proteins are present.

Examples of the G protein coupled receptor protein are human amygdaloid nucleus-derived G protein coupled receptor proteins

having an amino acid sequence selected from the group consisting of an amino acid sequence represented by SEQ ID NO: 34 and/or an amino acid sequence represented by SEQ ID NO: 35, etc. These G protein coupled receptor proteins may include

5 proteins wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are deleted from the amino acid sequence of SEQ ID NO: 34 or SEQ ID NO: 35, proteins wherein one or more amino acid residues (preferably from 2 to 30 amino acid

10 residues, more preferably from 2 to 10 amino acid residues) are added to the amino acid sequence of SEQ ID NO: 34 or SEQ ID NO: 35, proteins wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) in the amino acid sequence of

15 SEQ ID NO: 34 or SEQ ID NO: 35, are substituted with one or more amino acid residues, etc.

In still another embodiment of the present invention, these G protein coupled receptor proteins are those derived from all cells and tissues (e.g. amygdaloid nucleus, pituitary

20 body, pancreas, brain, kidney, liver, gonad, thyroid gland, cholecyst, bone marrow, lung, digestive duct, blood vessel, heart, thymus, leukocyte, etc.) of warm-blooded animals (e.g. guinea pig, rat, mouse, swine, sheep, cattle, monkey, human beings, etc.), and any of proteins as long as they

25 comprise an amino acid sequence represented by SEQ ID NO: 38, or substantial equivalents to the amino acid sequence represented by SEQ ID NO: 38, preferably an amino acid sequence represented by SEQ ID NO: 39, or substantial equivalents to the amino acid sequence represented by SEQ ID NO: 39.

30 These G protein coupled receptor proteins may include proteins having an amino acid sequence represented by SEQ ID NO: 38, proteins wherein the amino acid sequence thereof is about 90% to 99.9% homologous to an amino acid sequence represented by SEQ ID NO: 38 and the activity thereof is substantially

35 equivalent to the protein having an amino acid sequence represented by SEQ ID NO: 38 and the like.

These G protein coupled receptor proteins are preferably

proteins having an amino acid sequence represented by SEQ ID NO: 39, proteins wherein the amino acid sequence thereof is about 90% to 99.9% homologous to an amino acid sequence represented by SEQ ID NO: 39 and the activity thereof is substantially equivalent to the protein having an amino acid sequence represented by SEQ ID NO: 39, etc. The substantially equivalent activity may include ligand binding activity, signal information transmitting, etc. The term "substantially equivalent" or "substantial equivalent" means that the nature of the ligand binding activity and the like is equivalent. Therefore, it is allowable that even differences among grades such as ligand binding affinity grades and ligand binding activity grades and quantitative factors such as molecular sizes or weights of receptor proteins are present.

It is suggested by data that the mouse pancreatic β -cell strain, MIN6-derived receptor protein of the present invention (e.g., SEQ ID NO: 38 and SEQ ID NO: 39, or proteins encoded by pMAH2-17) is a novel purinoceptor subtype which is clearly distinct from prior art purinoceptors.

In another more specific embodiment of the present invention, G protein coupled receptor proteins include mouse pancreatic β -cell line, MIN6, derived G protein coupled receptor proteins comprising an amino acid sequence represented by SEQ ID NO: 38, mouse pancreatic β -cell line, MIN6, derived G protein coupled receptor proteins wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are deleted from the amino acid sequence of SEQ ID NO: 38, proteins wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are added to the amino acid sequence of SEQ ID NO: 38, proteins wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are substituted with other amino acid residues in the amino acid sequence of SEQ ID NO: 38, etc. Further preferably these G protein coupled receptor proteins include mouse pancreatic β -cell line, MIN6, derived G

protein coupled receptor proteins comprising an amino acid sequence represented by SEQ ID NO: 39, mouse pancreatic β -cell line, MIN6, derived G protein coupled receptor proteins wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are deleted from the amino acid sequence of SEQ ID NO: 39, proteins wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are added to the amino acid sequence of SEQ ID NO: 39, proteins wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) in the amino acid sequence of SEQ ID NO: 39 are substituted with other amino acid residues, etc.

15 In still another embodiment of the present invention, these G protein coupled receptor proteins are those derived from all cells and tissues (e.g. placenta, gonad, amygdaloid nucleus, pituitary body, pancreas, brain, kidney, liver, thyroid gland, cholecyst, bone marrow, lung, digestive duct, blood vessel, heart, thymus, leukocyte, etc.) of human beings, and any of proteins as long as they comprise an amino acid sequence represented by SEQ ID NO: 56, or substantial equivalents to the amino acid sequence represented by SEQ ID NO: 56. These G protein coupled receptor proteins may include proteins having an amino acid sequence represented by SEQ ID NO: 56, proteins wherein the amino acid sequence thereof is about 90% to 99.9% homologous to an amino acid sequence represented by SEQ ID NO: 56 and the activity thereof is substantially equivalent to the protein having an amino acid sequence represented by SEQ ID NO: 56 and the like. The substantially equivalent activity may include ligand binding activity, signal information transmitting, etc. The term "substantially equivalent" or "substantial equivalent" means that the nature of the ligand binding activity and the like is equivalent. Therefore, it is allowable that even differences among grades such as ligand binding affinity grades and ligand binding activity grades and quantitative factors

such as molecular sizes or weights of receptor proteins are present.

In another more specific embodiment of the present invention, G protein coupled receptor proteins include G protein coupled receptor proteins comprising an amino acid sequence represented by SEQ ID NO: 56, G protein coupled receptor proteins wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are deleted from the amino acid sequence of SEQ ID NO: 56, proteins wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are added to the amino acid sequence of SEQ ID NO: 56, proteins wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) in the amino acid sequence of SEQ ID NO: 56, are substituted with other amino acid residues, etc.

A portion of the amino acid sequence may be modified (e.g. addition, deletion, substitution with other amino acids, etc.) in the G protein coupled receptor proteins of the present invention.

Furthermore, the G protein coupled receptor proteins of the present invention includes those wherein N-terminal Met is protected with a protecting group (e.g., C₁₋₆ acyl group such as formyl, acetyl, etc.), those wherein the N-terminal side of Glu is cleaved in vivo to make said Glu pyroglutaminated, those wherein the intramolecular side chain of amino acids is protected with a suitable protecting group (e.g., C₁₋₆ acyl group such as formyl, acetyl, etc.), conjugated proteins such as so-called "glycoproteins" wherein saccharide chains are bonded, etc.

The salt of said G protein coupled receptor protein of the present invention includes preferably physiologically acceptable acid addition salts. Examples of such salts are salts thereof with inorganic acids (e.g. hydrochloric acid, phosphoric acid, hydrobromic acid, sulfuric acid, etc.), salts

thereof with organic acids (e.g. acetic acid, formic acid, propionic acid, fumaric acid, maleic acid, succinic acid, tartaric acid, citric acid, malic acid, oxalic acid, benzoic acid, methanesulfonic acid, benzenesulfonic acid, etc.), etc.

5 The G protein coupled receptor protein or its salt of the present invention may be manufactured from the tissues or cells of warm-blooded animals by purifying methods which are known per se by those skilled in the art or methods similar thereto or may be manufactured by culturing the transformant
10 (or transfectant) (as described herein below) containing G protein coupled receptor protein encoding DNA . The protein or its salt of the present invention may be manufactured by the peptide synthesis as described herein below.

15 The G protein coupled receptor protein fragment (the partial peptide of said G protein coupled receptor protein) may include, for example, the site which is exposed outside cell membranes, among the G protein coupled receptor protein molecule. Examples of the fragment are peptides containing a region which is analyzed as an extracellular area
20 (hydrophilic region or site) in a hydrophobic plotting analysis on the G protein coupled receptor protein represented by any of Figures 24, 25, 28, 31, 32, 36, 38, 41, 44, 47, 50, 53, 57, 58, 59, 64, 70, 74, and 78.

A peptide which partly contains a hydrophobic region or site
25 may be used as well. Further, a peptide which separately contains each domain may be used too although the partial peptide (peptide fragment) which contains plural domains at the same time will be used as well.

30 The salt of said G protein coupled receptor protein fragment (partial peptide thereof) includes preferably physiologically acceptable acid addition salts. Examples of such salts are salts thereof with inorganic acids (e.g. hydrochloric acid, phosphoric acid, hydrobromic acid, sulfuric acid, etc.), salts thereof with organic acids (e.g. acetic
35 acid, formic acid, propionic acid, fumaric acid, maleic acid, succinic acid, tartaric acid, citric acid, malic acid, oxalic acid, benzoic acid, methanesulfonic acid, benzenesulfonic

acid, etc.), etc.

The G protein coupled receptor protein fragment (the partial peptide of the G protein coupled receptor protein) may be manufactured by synthesizing methods for peptides which are known per se by those skilled in the art or methods similar thereto or by cleaving (digesting) G protein coupled receptor proteins by a suitable peptidase. Methods of synthesizing peptide may be any of a solid phase synthesis and a liquid phase synthesis. Thus, a partial peptide (peptide fragment) or amino acids which can construct the protein of the present invention is condensed with the residual part thereof and, when the product has a protective group, said protective group is detached whereupon a desired peptide can be manufactured. Examples of the known methods for condensation and for detachment of protective groups include the following ① to ⑤ :

- ① M. Bodanszky and M. A. Ondetti: Peptide Synthesis, Interscience Publishers, New York (1966).
- ② Schroeder and Luebke: The Peptide, Academic Press, New York, 1965.
- ③ Nobuo Izumiya et al.: Fundamentals and Experiments of the Peptide Synthesis, Maruzen KK, Japan (1975).
- ④ Haruaki Yajima and Shumpei Sakakibara: "Seikagaku Jikken Koza 1" (Experiments of Biochemistry, Part 1), "Tanpakusitu No Kagaku IV" (Chemistry of Protein, IV), p.205 (1977), Japan.
- ⑤ Haruaki Yajima (ed): Development of Pharmaceuticals (Second Series), Vol. 14, Peptide Synthesis, Hirokawa Shoten, Japan.

After the reaction, conventional purifying techniques such as salting-out, extraction with solvents, distillation, column chromatography, liquid chromatography, electrophoresis, recrystallization, etc. are optionally combined so that the protein of the present invention can be purified and isolated. When the protein obtained as such is a free compound, it may be

converted to a suitable salt by known methods while, when it is obtained as a salt, the salt may be converted to a free compound or other salt compounds by known methods.

5 Furthermore, the product may be manufactured by culturing the transformant (transfectant) containing the DNA coding for said partial peptide.

10 The G protein coupled receptor protein-encoding DNA obtained by the above-mentioned screening method using the DNA of the present invention and the G protein coupled receptor protein encoded by said DNA or the peptide fragment (partial peptide thereof) encoded by said DNA may, for example, be used for the determination of a ligand to said G protein coupled receptor protein or for the screening of a compound which inhibits the binding of said protein coupled receptor protein with a ligand.

15 In that case, an expression system for the G protein coupled receptor protein-encoding DNA is at first constructed. Hosts for said DNA may be any of animal cells, insect cells, yeasts, Bacillus subtilis, Escherichia coli, etc. , Promoters used therefor may be anyone so far as it is suitable as a promoter for the host used for gene expression. Incidentally, the utilization of enhancers for expression is effective as well.

25 Then the expressing cells per se which constructed to express the G protein coupled receptor protein or the cell membrane fractions prepared therefrom by methods known per se by those skilled in the art or methods similar thereto may be subjected to a variety of receptor binding experiments. Ligands used therefor may include any of compounds labeled by a commercially available radioisotope, etc., culture supernatants and tissue extracts which are directly labeled by a chloramine T method or by a lactoperoxidase method. Separation of bonded or free ligands may be carried out by a direct washing when cells adhered to substrates are used, 30 while, in the case of floating cells or cell membrane fractions thereof, it may be carried out by means of centrifugal separation or filtration. Nonspecific binding with container,

etc. may be estimated by addition of unlabeled ligands which are about 100 times as much concentrated relatively to the poured labeled ligand.

5 The ligand which is obtained by such a receptor binding experiment may be subjected to a discrimination of agonist versus antagonist.

10 To be more specific, a natural substance or compound which is presumed to be a ligand with the G protein coupled receptor protein-expressing cell is cultured and, after that, the culture supernatant liquid is collected or the cell is extracted. A change in the components contained therein is measured by, for example, a commercially available measuring kit (e.g. kits for cAMP, diacylglycerol, cGMP, proteinkinase A, etc.). Alternatively, it is possible to measure
15 physiological responses such as liberation of Fura-2, [³H]arachidonic acid and [³H]inositol phosphate metabolites by methods known per se by those skilled in the art or methods similar thereto. The compound or natural substance which is obtained by such a screening is an agonist for said G protein coupled receptor protein or an antagonist for said G protein coupled receptor protein and is presumed to act on the tissues and cells in which said receptor is distributed. Accordingly, it is possible to check the pharmaceutical response (pharmaceutical effect) more efficiently by referring
20 to the distribution disclosed (clarified) by a northern blotting or the like. Moreover, a development of compounds having a novel pharmaceutical response (pharmaceutical effect) in, for example, central nervous tissues, circulatory system, kidney, pancreas, etc. is expected. An efficient development of pharmaceuticals can be proceeded by amplifying G protein coupled receptor protein-encoding DNA selectively from tissues.

25 The G protein coupled receptor protein-encoding DNA of the present invention may be any coding DNA as long as it contains a nucleotide sequence coding for a G protein coupled receptor protein which contains an amino acid sequence
35 substantially equivalent to the amino acid sequence having SEQ ID NO: 24 and/or which has an activity substantially

equivalent to the amino acid sequence having SEQ ID NO: 24,
a G protein coupled receptor protein which contains an amino
acid sequence substantially equivalent to the amino acid
sequence having SEQ ID NO: 25 and/or which has an activity
substantially equivalent to the amino acid sequence having SEQ
ID NO: 25, a G protein coupled receptor protein which contains
an amino acid sequence substantially equivalent to the amino
acid sequence having SEQ ID NO: 26 and/or which has an activity
substantially equivalent to the amino acid sequence having SEQ
ID NO: 26, a G protein coupled receptor protein which contains
an amino acid sequence substantially equivalent to the amino
acid sequence having SEQ ID NO: 27 and/or which has an activity
substantially equivalent to the amino acid sequence having SEQ
ID NO: 27, or a G protein coupled receptor protein which
contains an amino acid sequence substantially equivalent to the
amino acid sequence having SEQ ID NO: 28 and/or which has an
activity substantially equivalent to the amino acid sequence
having SEQ ID NO: 28.

Still the G protein coupled receptor protein-encoding
DNA of the present invention may be any coding DNA as long as
it contains a nucleotide sequence coding for a G protein
coupled receptor protein which contains an amino acid sequence
substantially equivalent to the amino acid sequence having
SEQ ID NO: 34 and/or which has an activity substantially
equivalent to the amino acid sequence having SEQ ID NO: 34,
or a G protein coupled receptor protein which contains an amino
acid sequence substantially equivalent to the amino acid
sequence having SEQ ID NO: 35 and/or which has an activity
substantially equivalent to the amino acid sequence having SEQ
ID NO: 35.

Yet the G protein coupled receptor protein-encoding
DNA of the present invention may be any coding DNA as long as
it contains a nucleotide sequence coding for a G protein
coupled receptor protein which contains an amino acid sequence
substantially equivalent to the amino acid sequence having
SEQ ID NO: 38 and/or which has an activity substantially
equivalent to the amino acid sequence having SEQ ID NO: 38, or

preferably a G protein coupled receptor protein which contains an amino acid sequence substantially equivalent to the amino acid sequence having SEQ ID NO: 39 and/or which has an activity substantially equivalent to the amino acid sequence having SEQ ID NO: 39.

Yet the G protein coupled receptor protein-encoding DNA of the present invention may be any coding DNA as long as it contains a nucleotide sequence coding for a G protein coupled receptor protein which contains an amino acid sequence substantially equivalent to the amino acid sequence having SEQ ID NO: 56 and/or which has an activity substantially equivalent to the amino acid sequence having SEQ ID NO: 56, or preferably a G protein coupled receptor protein which contains an amino acid sequence substantially equivalent to the amino acid sequence having SEQ ID NO: 56 and/or which has an activity substantially equivalent to the amino acid sequence having SEQ ID NO: 56.

The DNA of the present invention may be any one of a human genome DNA, a human genome DNA library, a human tissue and cell-derived cDNA, a human tissue and cell-derived cDNA library and a synthetic DNA. The vector used for the library may include bacteriophage, plasmid, cosmid, phagemid, etc. The DNA can be further amplified directly by the reverse transcriptase polymerase chain reaction (hereinafter briefly referred to as "RT-PCR") using mRNA fractions prepared from tissues and cells.

In an embodiment, the DNA coding for the human pituitary gland-derived G protein coupled receptor protein comprising the amino acid sequence of SEQ ID NO: 24 includes DNA having a nucleotide sequence represented by SEQ ID NO: 29, etc. The DNA coding for the human pituitary gland-derived G protein coupled receptor protein comprising the amino acid sequence of SEQ ID NO: 25 includes DNA having a nucleotide sequence represented by SEQ ID NO: 30, etc. The DNA coding for the human pituitary gland-derived G protein coupled receptor protein comprising the amino acid sequence of SEQ ID NO: 26 includes DNA having a nucleotide sequence represented by

SEQ ID NO: 31, etc. The DNA coding for the mouse pancreas-derived G protein coupled receptor protein comprising the amino acid sequence of SEQ ID NO: 27 includes DNA having a nucleotide sequence represented by SEQ ID NO: 32, etc. The DNA coding for the mouse pancreas-derived G protein coupled receptor protein comprising the amino acid sequence of SEQ ID NO: 28 includes DNA having a nucleotide sequence represented by SEQ ID NO: 33, etc.

In another embodiment, the DNA coding for the human amygdaloid nucleus-derived G protein coupled receptor protein comprising the amino acid sequence of SEQ ID NO: 34 includes DNA having a nucleotide sequence represented by SEQ ID NO: 36, etc. The DNA coding for the human amygdaloid nucleus-derived G protein coupled receptor protein comprising the amino acid sequence of SEQ ID NO: 35 includes DNA having a nucleotide sequence represented by SEQ ID NO: 37, etc. The DNA coding for the human amygdaloid nucleus-derived G protein coupled receptor protein comprising the amino acid sequence of SEQ ID NO: 34 or the amino acid sequence of SEQ ID NO: 35 includes DNA having a nucleotide sequence represented by SEQ ID NO: 36, DNA having a nucleotide sequence represented by SEQ ID NO: 37, etc. Still in another embodiment, the DNA coding for the mouse pancreatic β -cell line, MIN6-derived G protein coupled receptor protein comprising the amino acid sequence of SEQ ID NO: 38 includes DNA having a nucleotide sequence represented by SEQ ID NO: 40, etc. The DNA coding for the mouse pancreatic β -cell line, MIN6-derived G protein coupled receptor protein comprising the amino acid sequence of SEQ ID NO: 39 includes DNA having a nucleotide sequence represented by SEQ ID NO: 41, etc. Yet in another embodiment, the DNA coding for the human-derived G protein coupled receptor protein comprising the amino acid sequence of SEQ ID NO: 56 includes DNA having a nucleotide sequence represented by SEQ ID NO: 57, etc.

The DNA completely coding for the G protein coupled receptor protein of the present invention can be cloned by (1) carrying out the PCR amplification using a synthetic DNA primer having a partial nucleotide sequence (nucleotide

fragment) of the G protein coupled receptor protein; or
(2) effecting the selection of a DNA constructed in a
suitable vector, based on the hybridization with a labeled
DNA fragment having part or all of the region encoding a human
G protein coupled receptor protein or a labeled synthetic DNA
having part or all of the coding region thereof.

The hybridization is carried out according to methods as
disclosed in, for example, Molecular Cloning, 2nd Ed., J.
Sambrook et al., Cold Spring Harbor Lab. Press, 1989.

When a DNA library commercially available in the market is
used, the hybridization is carried out according to protocols
manuals attached thereto.

The cloned G protein coupled receptor protein-
encoding DNA of the present invention can be used as it is, or
can be used, as desired, after modifications including
digestion with a restriction enzyme or addition of a linker
or adapter, etc. depending upon objects. The DNA may have
an initiation codon, ATG, on the 5' terminal side and
a termination codon, TAA, TGA or TAG, on the 3' terminal side.
These initiation and termination codons can be ligated by
using a suitable synthetic DNA adapter.

An expression vector for G protein coupled receptor
proteins can be produced by, for example, (a) cutting out a
target DNA fragment from the G protein coupled receptor
protein-encoding DNA of the present invention and (b) ligating
the target DNA fragment with the downstream site of a promoter
in a suitable expression vector.

The vector may include plasmids derived from
Escherichia coli (e.g., pBR322, pBR325, pUC12, pUC13, etc.),
plasmids derived from Bacillus subtilis (e.g., PUB110, PTP5,
pC194, etc.), plasmids derived from yeasts (e.g., pSH19, pSH15,
etc.), bacteriophages such as λ -phage, and animal virus such
as retrovirus, vaccinia virus and baculovirus.

According to the present invention, any promoter can
be used as long as it is compatible with a host which is used
for expressing a gene. When the host for the transformation is
E. coli, the promoters are preferably trp promoters, lac

promoters, recA promoters, λ_{PL} promoters, lpp promoters, etc. When the host for the transformation is the Bacillus, the promoters are preferably SPO1 promoters, SPO2 promoters, penP promoters, etc. When the host is an yeast, the promoters are preferably PHO5 promoters, PGK promoters, GAP promoters, ADH promoters, etc. When the host is an animal cell, the promoters include SV40-derived promoters, retrovirus promoters, metallothionein promoters, heat shock promoters, cytomegalovirus promoters, SR α promoters, etc. An enhancer can be effectively utilized for the expression.

As required, furthermore, a host-compatible signal sequence is added to the N-terminal side of the G protein coupled receptor protein. When the host is E. coli, the utilizable signal sequences may include alkaline phosphatase signal sequences, OmpA signal sequences, etc. When the host is the Bacillus, they may include α -amylase signal sequences, subtilisin signal sequences, etc. When the host is an yeast, they may include mating factor α signal sequences, invertase signal sequences, etc. When the host is an animal cell, they may include insulin signal sequences, α -interferon signal sequences, antibody molecule signal sequences, etc.

A transformant or transfectant is produced by using the vector thus constructed, which carries the G protein coupled receptor protein-encoding DNA of the present invention. The host may be, for example, Escherichia microorganisms, Bacillus microorganisms, yeasts, insect cells, animal cells, etc. Examples of the Escherichia and Bacillus microorganisms include Escherichia coli K12-DH1 [Proc. Natl. Acad. Sci. USA, Vol. 60, 160 (1968)], JM103 [Nucleic Acids Research, Vol. 9, 309 (1981)], JA221 [Journal of Molecular Biology, Vol. 120, 517 (1978)], HB101 [Journal of Molecular Biology, Vol. 41, 459 (1969)], C600 [Genetics, Vol. 39, 440 (1954)], etc. Examples of the Bacillus microorganism are, for example, Bacillus subtilis MI114 [Gene, Vol. 24, 255 (1983)], 207-21 [Journal of Biochemistry, Vol. 95, 87 (1984)], etc. The yeast may be, for example, Saccharomyces cerevisiae AH22, AH22R⁻, NA87-11A, DKD-5D, 20B-12, etc. The insect may include

a silkworm (Bombyx mori larva), [Maeda et al, Nature, Vol. 315, 592 (1985)] etc. The host animal cell may be, for example, monkey-derived cell line, COS-7, Vero, Chinese hamster ovary cell line (CHO cell), DHFR gene-deficient Chinese hamster cell line (dhfr⁻ CHO cell), mouse L cell, murine myeloma cell, human FL cell, etc.

Depending on the host cell used, transformation is done using standard techniques appropriate to such cells. Transformation of Escherichia microorganisms can be carried out in accordance with methods as disclosed in, for example, Proc. Natl. Acad. Sci. USA, Vol. 69, 2110 (1972), Gene, Vol. 17, 107 (1982), etc. Transformation of Bacillus microorganisms can be carried out in accordance with methods as disclosed in, for example, Molecular & General Genetics, Vol. 168, 111 (1979), etc. Transformation of the yeast can be carried out in accordance with methods as disclosed in, for example, Proc. Natl. Acad. Sci. USA, Vol. 75, 1929 (1978), etc. The insect cells can be transformed in accordance with methods as disclosed in, for example, Bio/Technology, 6, 47-55, 1988. The animal cells can be transformed by methods as disclosed in, for example, Virology, Vol. 52, 456, 1973, etc. The transformants or transfectants which are transformed with expression vectors containing a G protein coupled receptor protein-encoding DNA are produced according to the aforementioned techniques.

Cultivation of the transformant (transfectant) in which the host is Escherichia or Bacillus microorganism can be carried out suitably in a liquid culture medium. The culture medium may contains carbon sources, nitrogen sources, minerals, etc. necessary for growing the transformant. The carbon source may include glucose, dextrin, soluble starch, sucrose, etc. The nitrogen source may include organic or inorganic substances such as ammonium salts, nitrates, corn steep liquor, peptone, casein, meat extracts, bean-cakes, potato extracts, etc. Examples of the minerals may include calcium chloride, sodium dihydrogen phosphate, magnesium chloride, etc. It is further allowable to add yeasts, vitamins, growth-

promoting factors, etc. It is desired that the culture medium is pH from about 5 to about 8.

The Escherichia microorganism culture medium is preferably an M9 medium containing, for example, glucose and casamino acid (Miller, Journal of Experiments in Molecular Genetics), 431-433, Cold Spring Harbor Laboratory, New York, 1972. Depending on necessity, the medium may be supplemented with drugs such as 3β -indolyl acrylic acid in order to improve efficiency of the promoter. In the case of the Escherichia host, the cultivation is carried out usually at about 15 to 43 °C for about 3 to 24 hours. As required, aeration and stirring may be applied. In the case of the Bacillus host, the cultivation is carried out usually at about 30 to 40 °C for about 6 to 24 hours. As required, aeration and stirring may be also applied. In the case of the transformant in which the host is an yeast, the culture medium used may include, for example, a Burkholder minimum medium [Bostian, K.L. et al., Proc. Natl. Acad. Sci. USA, Vol. 77, 4505 (1980)], an SD medium containing 0.5% casamino acid [Bitter, G.A. et al., Proc. Natl. Acad. Sci. USA, Vol. 81, 5330 (1984)], etc. It is preferable that pH of the culture medium is adjusted to be from about 5 to about 8. The cultivation is carried out usually at about 20 to 35 °C for about 24 to 72 hours. As required, aeration and stirring may be applied.

In the case of the transformant in which the host is an insect, the culture medium used may include those obtained by suitably adding additives such as passivated (or immobilized) 10% bovine serum and the like to the Grace's insect medium (Grace, T.C.C., Nature, 195, 788 (1962)). It is preferable that pH of the culture medium is adjusted to be about 6.2 to 6.4. The cultivation is usually carried out at about 27 °C for about 3 to 5 days. As desired, aeration and stirring may be applied. In the case of the transformant in which the host is an animal cell, the culture medium used may include MEM medium [Science, Vol. 122, 501 (1952)], DMEM medium [Virology, Vol. 8, 396 (1959)], RPMI 1640 medium [Journal of the American Medical Association, Vol. 199, 519 (1967)], 199 medium [Proceedings of

the Society of the Biological Medicine, Vol. 73, 1 (1950)],
etc. which are containing, for example, about 5 to 20% of
fetal calf serum. It is preferable that the pH is from about
6 to about 8. The cultivation is usually carried out at about
5 30 to 40 °C for about 15 to 60 hours. As required, aeration
and stirring may be applied.

Separation and purification of the G protein coupled
receptor protein from the above-mentioned cultures can be
carried out according to methods described herein below.

10 To extract G protein coupled receptor proteins
from the cultured microorganisms or cells, the microorganisms
or cells are collected by known methods after the cultivation,
suspended in a suitable buffer solution, disrupted by
ultrasonic waves, lysozyme and/or freezing and thawing, etc.
15 and, then, a crude extract of the G protein coupled receptor
protein is obtained by centrifugation or filtration. Other
conventional extracting or isolating methods can be applied.
The buffer solution may contain a protein-denaturing agent
such as urea or guanidine hydrochloride or a surfactant such
20 as Triton X-100 (registered trademark, hereinafter often
referred to as "TM").

In case where G protein coupled receptor proteins
are secreted into culture media, supernatant liquids are
separated from the microorganisms or cells after the
25 cultivation is finished and the resulting supernatant liquid is
collected by widely known methods. The culture supernatant
liquid and extract containing G protein coupled receptor
proteins can be purified by suitable combinations of widely
known methods for separation, isolation and purification.
30 The widely known methods of separation, isolation and
purification may include methods which utilizes solubility,
such as salting out or sedimentation with solvents
methods which utilizes chiefly a difference in the molecular
size or weight, such as dialysis, ultrafiltration, gel
35 filtration and SDS-polyacrylamide gel electrophoresis, methods
utilizing a difference in the electric charge, such as
ion-exchange chromatography, methods utilizing specific

affinity such as affinity chromatography, methods utilizing a difference in the hydrophobic property, such as inverse-phase high-performance liquid chromatography, and methods utilizing a difference in the isoelectric point such as isoelectric electrophoresis, etc.

In case where the G protein coupled receptor protein thus obtained is in a free form, the free protein can be converted into a salt thereof by known methods or method analogous thereto. In case where the G protein coupled receptor protein thus obtained is in a salt form vice versa, the protein salt can be converted into a free form or into any other salt thereof by known methods or method analogous thereto.

The G protein coupled receptor protein produced by the transformant can be arbitrarily modified or a polypeptide can be partly removed therefrom, by the action of a suitable protein-modifying enzyme before or after the purification. The protein-modifying enzyme may include trypsin, chymotrypsin, arginyl endopeptidase, protein kinase, glycosidase, etc. The activity of the G protein coupled receptor protein thus formed can be measured by experimenting the coupling (or binding) with a ligand, or by enzyme immunoassays (enzyme linked immunoassays) using specific antibodies.

The G protein coupled receptor protein-encoding DNA and the G protein coupled receptor protein of the present invention can be used for:

- ① methods of determining ligands for the G protein coupled receptor protein of the present invention,
- ② obtaining an antibody and an antiserum,
- ③ constructing a system for expressing a recombinant receptor protein,
- ④ developing a receptor-binding assay system using the above developing system and screening pharmaceutical candidate compounds,
- ⑤ designing drugs based upon the comparison with ligands and receptors which have a similar or analogous structure,

- ⑥ preparing a probe in the analysis of genes and preparing a PCR primer, and
- ⑦ gene manipulating therapy.

In particular, it is allowable to screen a G protein coupled receptor agonist or antagonist specific to a warm-blooded animal such as human being by a receptor-binding assay system which uses a system for expressing a recombinant G protein coupled receptor protein of the present invention. The agonist or antagonist thus screened or characterized permits various applications including prevention and/or therapy of a variety of diseases.

Concretely described below are uses of G protein coupled receptor proteins, partial peptide thereof (peptide fragment thereof), G protein coupled receptor protein-encoding DNAs and antibodies against the G protein coupled receptor protein according to the present invention.

As hereunder, more detailed description will be made on the usefulness of the G protein coupled receptor protein-encoding DNA obtained by the screening method for G protein coupled receptor protein-encoding DNAs according to the present invention, the G protein coupled receptor proteins encoded by said DNA, peptide fragments or segments thereof (including partial peptides thereof) or salts thereof (hereinafter, those including their salts, will be referred to as the "G protein coupled receptor protein or a peptide fragment thereof"), cells or cell membrane fractions thereof each containing the recombinant type G protein coupled receptor protein, etc. Their various applications are also disclosed herein below.

(1) Method for Determining Ligands to the G Protein Coupled Receptor Protein

The G protein coupled receptor protein (or the peptide segment thereof) is useful as a reagent for investigating or determining a ligand to said G protein coupled receptor protein.

According to the present invention, methods for determining a ligand to the G protein coupled receptor protein

which comprises contacting the G protein coupled receptor protein or the peptide segment or fragment thereof with the compound to be tested are provided.

5 The compound to be tested may include not only known
ligands such as angiotensins, bombesins, canavanoids,
cholecystokinins, glutamine, serotonin, melatonins,
neuropeptides Y, opioids, purine, vasopressins, oxytocins,
VIP (vasoactive intestinal and related peptides),
somatostatins, dopamine, motilins, amylin, bradykinins,
10 CGRP (calcitonin gene related peptides), adrenomedullins,
leukotrienes, pancreastatins, prostaglandins, thromboxanes,
adenosine, adrenaline, α - and β -chemokines (IL-8, GRO α ,
GRO β , GRO γ , NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14,
MCP-3, I-309, MIP1 α , MIP-1 β , RANTES, etc.), endothelins,
15 enterogastrins, histamine, neurotensins, TRH, pancreatic
polypeptides, galanin, modified derivatives thereof, analogues
thereof, family members thereof and the like but also tissue
extracts, cell culture supernatants, etc. of warm-blooded
animals (such as mice, rats, swines, cattle, sheep, monkeys
20 and human being), etc. For example, said tissue extract, said
cell culture supernatant, etc. is added to the G protein
coupled receptor protein for measurement of the cell
stimulating activity, etc. and fractionated by relying on the
measurements whereupon a single ligand can be finally
25 obtained.

In one specific embodiment of the present invention,
said method for determining the ligand includes a method for
determining a compound or a salt thereof capable of
stimulating a target cell which comprises binding said compound
30 with the G protein coupled receptor protein either in the
presence of the G protein coupled receptor protein or the
peptide segment thereof or in a receptor binding assay system
in which the expression system for the recombinant type
receptor protein is constructed and used; and measuring the
35 receptor-mediated cell stimulating activity, etc.
Examples of said cell stimulating activities include promoting
activity or inhibiting activity on biological responses,

e.g. liberation of arachidonic acid, liberation of acetylcholine, liberation of endocellular Ca^{2+} , production of endocellular cAMP, production of endocellular cGMP, production of inositol phosphate, changes in the cell membrane potential, phosphorylation of endocellular protein, activation of c-fos, lowering in pH, activation of G protein, cell promulgation, etc. Examples of said compound or salt capable of stimulating the cell via binding with the G protein coupled receptor protein include peptides, proteins, nonpeptidic compounds, synthetic compounds, fermented products, etc.

In said method for determining the ligand, the characteristic feature is that when the G protein coupled receptor protein or the peptide segment thereof is contacted with the test compound, for example, the binding amount, the cell stimulating activity, etc. of the test compound to the G protein coupled receptor protein or the peptide segment thereof is measured.

In more specific embodiments of the present invention, said methods for determining the ligand includes:

- ① a method of determining a ligand to a G protein coupled receptor protein, which comprises contacting a labeled test compound with a G protein coupled receptor protein or a peptide segment thereof, and measuring the amount of the labeled test compound binding with said protein or salt thereof or with said peptide fragment or salt thereof;
- ② a method of determining a ligand to a G protein coupled receptor protein, which comprises contacting a labeled test compound with cells containing the G protein coupled receptor protein or the membrane fraction of said cell, and measuring the amount of the labeled test compound binding with said cells or said cell fraction;
- ③ a method of determining a ligand to a G protein coupled receptor protein, which comprises contacting a labeled test compound with the G protein coupled receptor protein expressed on cell membranes by culturing transformants containing the DNA coding for the G protein coupled receptor protein, and measuring the amount of the labeled test compound binding with

said G protein coupled receptor protein;

④ a method of determining a ligand to a G protein coupled receptor protein, which comprises contacting a test compound with cells containing the G protein coupled receptor protein, and measuring the cell stimulating activity (e.g. promoting or inhibiting activity on biological responses such as liberation of arachidonic acid, liberation of acetylcholine, liberation of endocellular Ca^{2+} , production of endocellular cAMP, production of endocellular cGMP, production of inositol phosphate, changes in the cell membrane potential, phosphorylation of endocellular protein, activation of c-fos, lowering in pH, activation of G protein, cell promulgation, etc.) via the G protein coupled receptor protein; and

⑤ a method of determining a ligand to the G protein coupled receptor protein, which comprises contacting a test compound with the G protein coupled receptor protein expressed on the cell membrane by culturing transformants containing the DNA coding for the G protein coupled receptor protein, and measuring the cell stimulating activity (activity for promoting or inhibiting physiological responses such as liberation of arachidonic acid, liberation of acetylcholine, liberation of endocellular Ca^{2+} , production of endocellular cAMP, production of endocellular cGMP, production of inositol phosphate, changes in the cell membrane potential, phosphorylation of endocellular protein, activation of c-fos, lowering in pH, activation of G protein, cell promulgation, etc.) via the G protein coupled receptor protein.

Described below are specific explanations on the determining method of ligands according to the present invention which are provided only for illustrative purposes.

First, the G protein coupled receptor protein used for the method for determining the ligand may include any material so far as it contains a G protein coupled receptor protein or a peptide fragment or segment thereof (including a partial peptide thereof) or a salt thereof although it is preferable to express a large amount of G protein coupled receptor proteins in animal cells.

In the manufacture of the G protein coupled receptor protein, the above-mentioned method can be used and it may be carried out by expressing said protein encoding DNA in mammalian cells or in insect cells. With respect to the DNA
5 fragment coding for the aimed region, complementary DNA may be used although it is not limited thereto. For example, gene fragments or synthetic DNA may be used as well.

In order to introduce the G protein coupled receptor protein-encoding DNA fragment into host animal cells and to
10 express it efficiently, it is preferred that said DNA fragment is incorporated into the downstream site of polyhedron promoters derived from nuclear polyhedrosis virus belonging to baculovirus, promoters derived from SV40, promoters derived from retrovirus, metallothionein promoters,
15 human heat shock promoters, cytomegalovirus promoters, SR α promoters, etc. Examinations of the quantity and the quality of the expressed receptor can be carried out by methods per se known to those of skill in the art or methods similar thereto. For example, they may be conducted by methods
20 described in publications such as Nambi, P. et al: The Journal of Biochemical Society, vol.267, pages 19555-19559 (1992).

Accordingly, with respect to the determination of the ligand, the material containing a G protein coupled receptor protein or peptide segment thereof may include
25 products containing G protein coupled receptor proteins which are purified by methods per se known to those of skill in the art or methods similar thereto, peptide fragments of said G protein coupled receptor protein, cells containing said G protein coupled receptor protein, membrane fractions of the
30 cell containing said protein, etc.

When the G protein coupled receptor protein-containing cell is used in the determining method of the ligand, said cell may be immobilized with binding agents including glutaraldehyde, formalin, etc. The immobilization
35 may be carried out by methods per se known to those of skill in the art or methods similar thereto.

The G protein coupled receptor protein-

containing cells are host cells expressing the G protein coupled receptor protein. Examples of said host cells are microorganisms such as Escherichia coli, Bacillus subtilis, yeasts, insect cells, animal cells, etc.

5 The cell membrane fraction is a cell membrane-rich fraction which is prepared by methods per se known to those of skill in the art or methods similar thereto after disruption of cells. Examples of cell disruption may include a method for squeezing cells using a Potter-Elvehjem homogenizer,
10 a disruption by a Waring blender or a Polytron (manufactured by Kinematica), a disruption by ultrasonic waves, a disruption via blowing out cells from small nozzles together with applying a pressure using a French press or the like, etc. In the fractionation of the cell membrane, a fractionation method by
15 means of centrifugal force such as a fractional centrifugal separation and a density gradient centrifugal separation is mainly used. For example, disrupted cellular liquid is centrifuged at a low speed (500 rpm to 3,000 rpm) for a short period (usually, from about one to ten minutes), the
20 supernatant liquid is further centrifuged at a high speed (1,500 rpm to 3,000 rpm) usually for 30 minutes to two hours and the resulting precipitate is used as a membrane fraction. Said membrane fraction contains a lot of the expressed G protein coupled receptor protein and a lot of membrane
25 components such as phospholipids and membrane proteins derived from the cells.

 The amount of the G protein coupled receptor protein in the membrane fraction cell containing said G protein coupled receptor protein is preferably 10^3 - 10^8 molecules
30 per cell or, suitably, 10^5 to 10^7 molecules per cell. Incidentally, the more the expressed amount, the higher the ligand binding activity (specific activity) per membrane fraction whereby the construction of a highly sensitive screening system becomes possible and, moreover, it may enable
35 us to measure the large amount of samples within the same lot.

 In conducting the above-mentioned methods ① to ② wherein ligands capable of binding with the G protein coupled

receptor protein are determined, a suitable G protein coupled receptor fraction and a labeled test compound are necessary. The G protein coupled receptor fraction is preferably a naturally occurring (natural type) G protein coupled receptor, a recombinant type G protein coupled receptor having the activity equivalent to that of the natural type. Here, the term "activity equivalent to" means the equivalent ligand binding activity, etc.

Suitable examples of the labeled test compound are angiotensin, bombesin, canavanin, cholecystokinin, glutamine, serotonin, melatonin, neuropeptide Y, opioid, purine, vasopressin, oxytocin, VIP (vasoactive intestinal and related peptides), somatostatin, dopamine, motilin, amylin, bradykinin, CGRP (calcitonin gene related peptides), adrenomedullin, leukotriene, pancreastatin, prostaglandin, thromboxane, adenosine, adrenaline, α - and β -chemokine (IL-8, GRO α , GRO β , GRO γ , NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, MIP1 α , MIP-1 β , RANTES, etc.), endothelin, enterogastrin, histamine, neurotensin, TRH, pancreatic polypeptides, galanin, an analogue derivative thereof, etc. which are labeled with [^3H], [^{125}I], [^{14}C], [^{35}S], etc.

Specifically, the determination of ligands capable of binding with G protein coupled receptor proteins is carried out as follows:

First, cells or cell membrane fractions containing the G protein coupled receptor protein are suspended in a buffer suitable for the determining method to prepare the receptor sample in conducting the method of determining the ligand binding with the G protein coupled receptor protein. The buffer may include any buffer such as Tris-HCl buffer or phosphate buffer with pH 4-10 (preferably, pH 6-8), etc., as long as it does not inhibit the binding of the ligand with the receptor. In addition, surface-active agents such as CHAPS, Tween 80TM (Kao-Atlas, Japan), digitonin, deoxycholate, etc. and various proteins such as bovine serum albumin (BSA), gelatin, milk derivatives, etc. may be added to the buffer with an object of decreasing the non-specific binding.

Further, a protease inhibitor such as PMSF, leupeptin, E-64 (manufactured by Peptide Laboratory), pepstatin, etc. may be added with an object of inhibiting the decomposition of the receptor and the ligand by protease. A test compound labeled with a predetermined (or certain) amount (5,000 cpm to 500,000 cpm) of [^3H], [^{125}I], [^{14}C], [^{35}S], etc. is made copresent in 0.01 ml to 10 ml of said receptor solution.

In order to know the non-specific binding amount (NSB), a reaction tube to which a great excessive amount of the unlabeled test compound is added is prepared as well.

The reaction is carried out at 0-50°C, (preferably at 4-37°C) for 20 minutes to 24 hours (preferably 30 minutes to three hours). After the reaction, it is filtered through a glass fiber filter or the like, washed with a suitable amount of the same buffer and the radioactivity remaining in the glass fiber filter is measured by means of a liquid scintillation counter or a gamma-counter. The test compound in which the count (B - NSB) obtained by subtracting the non-specific binding amount (NSB) from the total binding amount (B) is more than 0 cpm can be selected as a ligand to the G protein coupled receptor protein of the present invention.

In conducting the above-mentioned methods ④ to ⑤ wherein ligands capable of binding with the G protein coupled receptor protein are determined, the cell stimulating activity (e.g. the liberation of arachidonic acid, the liberation of acetylcholine, endocellular Ca^{2+} liberation, endocellular cAMP production, the production of inositol phosphate, changes in the cell membrane potential, the phosphorylation of endocellular protein, the activation of c-fos, lowering of pH, the activation of G protein, cell promulgation, etc.) mediated by the G protein coupled receptor protein may be measured by known methods or by the use of commercially available measuring kits. To be more specific, G protein coupled receptor protein-containing cells are at first cultured in a multi-well plate or the like.

In conducting the determination of ligand, it is substituted with a fresh medium or a suitable buffer which

does not show toxicity to the cells in advance of the experiment, and incubated for certain period after adding a test compound, etc. thereto. Then, the cells are extracted or the supernatant liquid is recovered and the resulting product is determined by each of the methods. When it is difficult to identify the production of the substance (e.g. arachdonic acid) which is to be an index for the cell stimulating activity due to the decomposing enzyme contained in the cell, an assay may be carried out by adding an inhibitor against said decomposing enzyme. With respect to the activity such as an inhibitory action against CAMP production, it may be detected as an inhibitory action against the production of the cells whose fundamental production is increased by forskolin or the like.

The kit used for the method of determining the ligand binding with the G protein coupled receptor protein includes a G protein coupled receptor protein or a peptide fragment thereof, cells containing the G protein coupled receptor protein, a membrane fraction from the cells containing the G protein coupled receptor protein, etc.

Examples of the kit for determining the ligand are as follows:

1. Reagent for Determining the Ligand.

① Buffer for Measurement and Buffer for Washing.

The buffering product wherein 0.05% of bovine serum albumin (manufactured by Sigma) is added to Hanks' Balanced Salt Solution (manufactured by Gibco).

This product may be sterilized by filtration through a membrane filter with a $0.45 \mu m$ pore size, and stored at $4^{\circ}C$ or may be formulated upon use.

② G Protein Coupled Receptor Protein Sample.

CHO cells in which G protein coupled receptor proteins are expressed are subcultured at the rate of 5×10^5 cells/well in a 12-well plate and cultured at $37^{\circ}C$ in a humidified 5% CO_2 /95% air atmosphere for two days to prepare the sample.

③ Labeled Test Compound.

The compound which is labeled with commercially

available [^3H], [^{125}I], [^{14}C], [^{35}S], etc. or labeled with a suitable method.

The product in a state of an aqueous solution is stored at 4°C or at -20°C and, upon use, diluted to $1\ \mu\text{M}$ with a buffer for the measurement. In the case of the test compound which is hardly soluble in water, it is dissolved in dimethylformamide, DMSO, methanol, etc.

④ Unlabeled Test Compound.

The same compound for the labeled one is prepared in a concentration of 100 to 1,000-fold concentrated state.

2. Method of Measurement.

① G protein coupled receptor protein-expressing CHO cells cultured in a 12-well tissue culture plate are washed twice with 1 ml of buffer for the measurement and then $490\ \mu\text{l}$ of buffer for the measurement is added to each well.

② Five μl of the labeled test compound is added and the mixture is made to react at room temperature for one hour. For measuring the nonspecific binding amount, $5\ \mu\text{l}$ of the unlabeled test compound is added.

③ The reaction solution is removed from each well, which is washed with 1 ml of a buffer for the measurement three times. The labeled test compound which is binding with the cells is dissolved in 0.2N NaOH -1% SDS and mixed with 4 ml of a liquid scintillator A (manufactured by Wako Pure Chemical, Japan).

④ Radioactivity is measured using a liquid scintillation counter (manufactured by Beckmann).

The ligand which can bind with the G protein coupled receptor protein include substances occurring or existing, for example, in brain, pituitary gland, pancreas, etc. Examples of the ligand are angiotensin, bombesin, canavaninoid, cholecystokinin, glutamine, serotonin, melatonin, neuropeptide Y, opioid, purine, vasopressin, oxytocin, VIP (vasoactive intestinal and related peptide), somatostatin, dopamine, motilin, amylin, bradykinin, CGRP (calcitonin gene related

peptide), adrenomedullin, leukotriene, pancreastatin, prostaglandin, thromboxane, thromboxatin, adenosine, adrenaline, α - and β -chemokine (IL-8, GRO α , GRO β , GRO γ , NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, MIP1 α , MIP-1 β , RANTES, etc.), endothelin, enterogastrin, histamine, neurotensin, TRH, pancreatic polypeptide, galanin, modified derivatives thereof, analogues thereof, etc.

Since the receptor protein encoded by pMAH2-17 is highly homologous to prinoceptors, it is considered that there are strong possibility of a subtype within prinoceptor families. All data including electrophysiological measurements are supporting that the mouse pancreatic β -cell strain, MIN6-derived receptor protein of the present invention (e.g., SEQ ID NO: 38 and SEQ ID NO: 39, or proteins encoded by pMAH2-17) is a novel purinoceptor subtype. In other words, it is suggested that the ligand capable of binding with the mouse pancreatic β -cell strain, MIN6-derived receptor protein of the present invention (e.g., SEQ ID NO: 38 and SEQ ID NO: 39, or proteins encoded by pMAH2-17) is a purine compound such as ATP. Further, the receptor protein (e.g., SEQ ID NO: 56, or proteins encoded by pMAH2-17) is considered to be a novel human type purinoceptor. It is presumed that it is advantageously useful in efficiently screening for agonists or antagonists to receptor proteins which control or regulate functions in the central nervous system or immune system, related to purine compounds, and in developing pharmaceuticals.

(2) Preventive and Therapeutic Agent for of G Protein Conjugated Receptor Protein Deficiency Diseases

If a ligand to the G protein coupled receptor protein is disclosed via the aforementioned method (1), the G protein coupled receptor protein-encoding DNA can be used a preventive and/or therapeutic agent for treating said G protein coupled receptor protein deficiency diseases depending upon the action that said ligand exerts.

For example, when there is a patient for whom the physiological action of the ligand cannot be expected because

of a decrease in the G protein coupled receptor protein in vivo, the amount of the G protein coupled receptor protein in the brain cells of said patient can be increased whereby the action of the ligand can be fully achieved by:

- 5 (a) administering the G protein coupled receptor protein-encoding DNA to the patient to express it; or
(b) inserting the G protein coupled receptor protein-encoding DNA into brain cells or the like to express it, followed by transplanting said brain cells or the like to said patient.

10 Accordingly, the G protein coupled receptor protein-encoding DNA can be used as a safe and less toxic preventive and therapeutic agent for the G protein coupled receptor protein deficiency diseases. In an embodiment, it is suggested that the ligands capable of binding with the mouse pancreatic
15 β -cell strain, MIN6-derived receptor protein of the present invention (e.g., SEQ ID NO: 38 and SEQ ID NO: 39, or proteins encoded by pMAH2-17) and further with the human-derived receptor protein of the present invention (e.g., SEQ ID NO: 56, or proteins encoded by pH2-17) are purine compounds such as
20 ATP. Therefore, the disease to be treated may include diseases or syndromes in connection with purine ligand compounds. Examples of such diseases may include cancer, immunodeficiency, autoimmune disease, rheumatoid arthritis, rejection on internal organ transplant, hypertension, diabetes, cystic fibrosis,
25 hypotension, incontinence of urine, pain, etc.

(3) Preventive and Therapeutic Pharmaceutical Composition for
Human-Derived G Protein Conjugated Receptor Protein
Deficiency Diseases

If the human-derived G protein coupled receptor
30 protein-encoding DNA is screened and a ligand for said human-derived G protein coupled receptor protein can be clarified using the above-mentioned method (1), the human-derived G protein coupled receptor protein-encoding DNA can be used as an agent for the prevention or therapy of the deficiency
35 diseases of said human-derived G protein coupled receptor protein depending upon the action that said ligand exhibits.

For example, when there is a patient for whom the physiological action of the ligand cannot be expected because of a decrease in the G protein coupled receptor protein in vivo, the amount of the G protein coupled receptor protein in the brain cells of said patient can be increased whereby the action of the ligand can be fully achieved by:

- (a) administering the G protein coupled receptor protein-encoding DNA to the patient to express it; or
- (b) inserting the G protein coupled receptor protein-encoding DNA into brain cells or the like to express it, followed by transplanting said brain cells or the like to said patient. Accordingly, the G protein coupled receptor protein-encoding DNA can be used as a safe and less toxic preventive and therapeutic agent for the G protein coupled receptor protein deficiency diseases.

When the G protein coupled receptor protein-encoding DNA is used as the above-mentioned agent, said DNA may be used alone or after inserting it into a suitable vector such as retrovirus vector, adenovirus vector, adenovirus-associated virus vector, etc. followed by subjecting the product vector to a conventional means. Thus, it may be administered orally parenterally, by inhalation spray, rectally, or topically as pharmaceutical compositions or formulations. Oral formulations include tablets (sugar-coated if necessary), capsules, elixirs, microcapsules, etc. Parenteral formulations include injections such as an aseptic solution or a suspension in water or in other pharmaceutically acceptable liquid. For example, the DNA of the present invention is admixed in a unit dose form which is required for preparing generally approved pharmaceutical preparations together with a physiologically acceptable carriers, flavoring agents, adjuvants, excipients, diluents, fillers, vehicles, antiseptics, stabilizers, binders, etc. whereupon the preparation can be manufactured. The amount of the effective component in those preparations is to be in such an extent that the suitable dose within an indicated range is achieved.

Examples of the additives which can be admixed in the

tablets, capsules, etc. are binders such as gelatin, corn starch, tragacanth and gum arabicum; fillers such as crystalline cellulose; swelling agents such as corn starch, gelatin and alginic acid; lubricating agents such as magnesium stearate; sweetening agents such as sucrose, lactose and saccharine; and flavoring agents such as pepper mint, akamono oil and cherry. When the unit dose form of the preparation is a capsule, a liquid carrier such as fat/oil may be further added in addition of the above-mentioned types of materials.

The aseptic composition for injection may be formulated by conventional practices for the preparations such as that the active substance in a vehicle such as water for injection is dissolved or suspended in naturally occurring plant oil such as sesame oil and palm oil.

Examples of an aqueous liquid for injection are a physiological saline solution and isotonic solutions containing glucose and other auxiliary agents (e.g. D-sorbitol, D-mannitol, sodium chloride, etc.) wherein a suitable auxiliary solubilizers such as alcohol (e.g. ethanol, etc.), polyalcohol (e.g. propylene glycol polyethylene glycol, etc.), nonionic surface-active agent (e.g. Polysorbate 80TM, HCO-50, etc.), etc. may be jointly used. Examples of an oily liquid include sesame oil, soybean oil, etc. wherein benzyl benzoate, benzyl alcohol, etc. may be jointly used as auxiliary solubilizers. In addition, buffers (e.g. phosphate buffer, sodium acetate buffer, etc.), analgesic agents (e.g. benzalkonium chloride, procaine hydrochloride, etc.), stabilizers (e.g. human serum albumin, polyethylene glycol, etc.), stabilizers (e.g. benzyl alcohol phenol, etc.), antioxidants, etc. may be admixed therewith too.

The prepared injection solution is filled in suitable ampoules. The preparation prepared as such is safe and less toxic and, therefore, it can be administered to warm-blooded animals (e.g., rat, rabbit, sheep, swine, cattle, cat, dog, monkey, human beings, etc.).

Specific dose levels of said DNA may vary depending upon a variety of factors including the activity of drugs employed, the age, body weight, general health, sex, diet,

time of administration, route of administration, drug combination, and the severity of the symptom. In the case of oral administration, it is usually about 0.1-100 mg, preferably about 1.0-50 mg or, more preferably, about 1.0-20 mg per day for adults (as 60 kg). When it is administered parenterally, its dose at a time may vary depending upon the object (patient) to be administered, organs to be administered, symptoms, administering methods, etc. but, in the case of injections, it is usually convenient to give by an intravenous route in an amount of about 0.01-30 mg, preferably about 0.1-20 mg or, more preferably, about 0.1-10mg per day to adults (as 60 kg). In the case of other animals, the dose calculated for 60 kg may be administered as well.

(4) Quantitative Determination of Ligand to the G Protein Conjugated Receptor Protein of the Present Invention.

The G protein coupled receptor protein or a peptide fragment thereof has a binding property to ligand and, therefore, it is capable of determining quantitatively an amount of ligands in vivo with good sensitivity.

This quantitative determination may be carried out by, for example, combining with a competitive method. Thus, samples to be determined is contacted with G protein coupled receptor proteins or peptide fragments thereof so that the ligand concentration in said sample can be determined.

In one embodiment of the quantitative determination, the protocols described in the following ① and ② or the methods similar thereto may be used:

① Hiroshi Irie (ed): "Radioimmunoassay" (Kodansha, Japan, 1974); and

② Hiroshi Irie (ed): "Radioimmunoassay, Second Series" (Kodansha, Japan, 1979).

(5) Screening of Compound Inhibiting the Binding of Ligand with the G Protein Conjugated Receptor Protein of the Present Invention.

G Protein coupled receptor proteins or peptide

fragments thereof are used. Alternatively, expression systems for recombinant type G Protein coupled receptor proteins or peptide fragments thereof are constructed and receptor binding assay systems using said expression system are used. In these assay systems, it is possible to screen compounds (e.g. peptides, proteins, nonpeptidic compounds, synthetic compounds, fermented products, cell extracts, plant extracts, animal tissue extracts, etc.) or salts thereof which inhibits the binding of a ligand with the G protein coupled receptor protein. Such a compound includes a compound exhibiting a G protein coupled receptor-mediated cell stimulating activity (e.g. activity of promoting or activity of inhibiting physiological reactions including liberation of arachdonic acid, liberation of acetylcholine, endocellular Ca^{2+} liberation, endocellular cAMP production, endocellular cGMP production, production of inositol phosphate, changes in cell membrane potential, phosphorylation of endocellular proteins, activation of c-fos, lowering of pH, activation of G protein, cell promulgation, etc.) (so-called "G protein coupled receptor-agonist"), a compound free of such a cell stimulating activity (so-called "G protein coupled receptor-antagonist"), etc.

Thus, the present invention provides a method of screening a compound which inhibits the binding of a ligand with a G protein coupled receptor protein or a salt thereof, characterized in comparing the following two cases:

- (i) the case wherein the ligand is contacted with the G protein coupled receptor protein or salt thereof, or a peptide fragment thereof or a salt thereof; and
- (ii) the case wherein the ligand is contacted with a mixture of the G protein coupled receptor protein or salt thereof or the peptide fragment or salt thereof and said test compound.

In said screening method, one characteristic feature of the present invention resides in that the amount of the ligand bonded with said G protein coupled receptor protein or the peptide fragment thereof, the cell stimulating activity of the ligand, etc. are measured in the case where (i) the

ligand is contacted with G protein coupled receptor proteins or peptide fragments thereof and in the case where (ii) the ligand and the test compound are contacted with the G protein coupled receptor protein or the peptide fragment thereof, respectively and then compared therebetween.

In one more specific embodiment of the present invention, the following is provided:

① a method of screening a compound or a salt thereof which inhibits the binding of a ligand with a G protein coupled receptor protein, characterized in that, when a labeled ligand is contacted with a G protein coupled receptor protein or a peptide fragment thereof and when a labeled ligand and a test compound are contacted with a G protein coupled receptor protein or a peptide fragment thereof, the amounts of the labeled ligand bonded with said protein or peptide fragment thereof or salt thereof are measured and compared;

② a method of screening a compound or a salt thereof which inhibits the binding of a ligand with a G protein coupled receptor protein, characterized in that, when a labeled ligand is contacted with cells containing G protein coupled receptor proteins or a membrane fraction of said cells and when a labeled ligand and a test compound are contacted with cells containing G protein coupled receptor proteins or a membrane fraction of said cells, the amounts of the labeled ligand binding with said protein or peptide fragment thereof or salt thereof are measured and compared;

③ a method of screening a compound or a salt thereof which inhibits the binding of a ligand with a G protein coupled receptor protein, characterized in that, when a labeled ligand is contacted with G protein coupled receptor proteins expressed on the cell membrane by culturing a transformant containing a G protein coupled receptor protein encoding DNA and when a labeled ligand and a test compound are contacted with G protein coupled receptor proteins expressed on the cell membrane by culturing a transformant containing a G protein coupled receptor protein encoding DNA, the amounts of the labeled ligand binding with said G protein coupled receptor

protein are measured and compared;

④ a method of screening a compound or a salt thereof which inhibits the binding of a ligand with a G protein coupled receptor protein, characterized in that, when a G protein coupled receptor protein-activating compound (e.g. a ligand to the G protein coupled receptor protein) is contacted with cells containing G protein coupled receptor proteins and when the G protein coupled receptor protein-activating compound and a test compound are contacted with cells containing G protein coupled receptor proteins, the resulting G protein coupled receptor protein-mediated cell stimulating activities (e.g. activities of promoting or activities of inhibiting physiological responses including liberation of arachdonic acid, liberation of acetylcholine, endocellular Ca^{2+} liberation, endocellular cAMP production, endocellular cGMP production, production of inositol phosphate, changes in cell membrane potential, phosphorylation of endocellular proteins, activation of c-fos, lowering of pH, activation of G protein, cell promulgation, etc.) are measured and compared; and

⑤ a method of screening a compound or a salt thereof which inhibits the binding of a ligand with a G protein coupled receptor protein, characterized in that, when a G protein coupled receptor protein-activating compound (e.g. a ligand to the G protein coupled receptor protein) is contacted with G protein coupled receptor proteins expressed on cell membranes by culturing transformants containing G protein coupled receptor protein-encoding DNA and when a G protein coupled receptor protein-activating compound and a test compound are contacted with the G protein coupled receptor protein expressed on the cell membrane by culturing the transformant containing the G protein coupled receptor protein-encoding DNA, the resulting G protein coupled receptor protein-mediated cell stimulating activities (activities of promoting or activities of inhibiting physiological responses such as liberation of arachdonic acid, liberation of acetylcholine, endocellular Ca^{2+} liberation, endocellular cAMP production, endocellular cGMP production, production of inositol phosphate, changes in cell

membrane potential, phosphorylation of endocellular proteins, activation of c-fos, lowering of pH, activation of G protein, and cell promulgation) are measured and compared.

Before the G protein coupled receptor protein of the present invention was obtained, the G protein coupled receptor agonist or antagonist had to be screened by, first, obtaining a candidate compound by using G protein coupled receptor protein-containing cells, tissues or cell membrane fractions derived from rat or the like (primary screening) and, then, making sure whether the candidate compound really inhibits the binding between human G protein coupled receptor proteins and ligands (secondary screening). Other receptor proteins inevitably exist when the cells, the tissues or the cell membrane fractions are used as they are, whereby they intrinsically make it difficult to screen agonists or antagonists to the desired receptor proteins. By using the human-derived G protein coupled receptor protein, however, there is no need of effecting the primary screening, whereby it is allowable to efficiently screen a compound that inhibits the binding between a ligand and a G protein coupled receptor. Besides, it is allowable to evaluate whether the compound that is screened is a G protein coupled receptor agonist or a G protein coupled receptor antagonist.

Specific explanations of the screening method will be given as hereunder.

First, with respect to the G protein coupled receptor protein used for the screening method of the present invention, any product may be used so far as it contains G protein coupled receptor proteins or peptide fragment thereof although the use of a membrane fraction of mammalian organs is suitable. However, human organs is extremely hardly available and, accordingly, G protein coupled receptor proteins which are expressed in a large amount using a recombinant are suitable for the screening.

In the manufacture of the G protein coupled receptor protein, the above-mentioned method can be used and it may be carried out by expressing the DNA coding for said protein in

mammalian cells or in insect cells. With respect to the DNA fragment coding for the target region, complementary DNA may be used although it is not limited thereto. Thus, for example, gene fragments or synthetic DNA may be used as well.

5 In order to introduce the G protein coupled receptor protein-encoding DNA fragment into host animal cells and to express it efficiently, it is preferred that said DNA fragment is incorporated into the downstream of polyhedron promoter of nuclear polyhedrosis virus belonging to baculovirus, promoter
10 derived from SV40, promoter of retrovirus, metallothionein promoter, human heat shock promoter, cytomegalovirus promoter, SR α promoter, etc. Examinations of the quantity and the quality of expressed receptors can be carried out by known methods per se or modified methods substantially analogous
15 thereto. For example, they may be conducted by the method described in publications such as Nambi, P. et al.: The Journal of Biochemical Society, vol.267, pages 19555-19559 (1992).

20 Accordingly, in the screening method, the substance containing a G protein coupled receptor protein or a peptide fragment thereof may be a G protein coupled receptor protein which is purified by known methods per se or a G protein coupled receptor protein fragment which is purified by known methods per se, or a cell containing said protein or a cell
25 membrane fraction of the cell containing said protein, etc.

When the G protein coupled receptor protein-containing cells are used in the screening method, said cells may be immobilized with glutaraldehyde, formalin, etc. The immobilization may be carried out by known methods per se
30 or modified methods substantially analogous thereto.

The G protein coupled receptor protein-containing cells are host cells expressing the G protein coupled receptor protein. Examples of said host cells may include Escherichia coli, Bacillus subtilis, yeasts, insect cells,
35 animal cells such as CHO cell and COS cell, etc.

Cell membrane fractions are fractions which contain a lot of cell membranes prepared by known methods per se or

modified methods substantially analogous thereto after disrupting or crushing the cells. Examples of disruptions of the cell may include methods by squeezing the cells with a Potter-Elvehjem homogenizer, disrupting or crushing by a Waring blender or a Polytron (manufactured by Kinematica), disrupting or crushing by means of ultrasonic wave, disrupting by blowing out the cells from small nozzles together with applying a pressure with a French press or the like, etc.

Fractionation of the cell membrane is carried out mainly by fractionation techniques by means of centrifugal force such as a fractional centrifugal separation and a density gradient centrifugal separation. For example, disrupted liquid of cells is centrifuged at a low speed (500 rpm to 3,000 rpm) for a short period (usually, from about one to ten minutes), the supernatant liquid is further centrifuged at a high speed (1,500 rpm to 3,000 rpm) usually for 30 minutes to two hours and the resulting precipitate is used as a membrane fraction. Said membrane fraction contains a lot of expressed G protein coupled receptor proteins and membrane components such as phospholipids and membrane proteins derived from the cells.

The amount of the G protein coupled receptor protein in the G protein coupled receptor protein-containing cell and in the cell membrane fraction obtained from the cell is preferably 10^3 - 10^8 molecules per cell or, suitably, 10^5 to 10^7 molecules per cell. Incidentally, the more the expressed amount, the higher the ligand binding activity (specific activity) per membrane fraction whereby the construction of a highly sensitive screening system is possible and, moreover, it is possible to measure the large amount of samples in the same lot.

In conducting the above-mentioned methods ① to ③ for screening the compound capable of inhibiting the binding of the ligand with the G protein coupled receptor protein, a suitable G protein coupled receptor fraction and a labeled ligand are necessary. With respect to the G protein coupled receptor fraction, it is preferred to use naturally occurring G protein coupled receptors (natural type G protein coupled

receptors) or recombinant type G protein coupled receptor fractions with the activity equivalent to that of the natural type G protein coupled. Here the term "activity equivalent to" means the same ligand binding activity, or the substantially equivalent ligand binding activity.

With respect to the labeled ligand, it is possible to use labeled ligands, labeled ligand analogized compounds, etc. For example, ligands labeled with [^3H], [^{125}I], [^{14}C], [^{35}S], etc. and other labeled substances may be utilized.

Specifically, G protein coupled receptor protein-containing cells or cell membrane fractions are first suspended in a buffer which is suitable for the determining method to prepare the receptor sample in conducting the screening for a compound which inhibits the binding of the ligand with the G protein coupled receptor protein.

With respect to the buffer, any buffer such as Tris-HCl buffer or phosphate buffer of pH 4-10 (preferably, pH 6-8) which does not inhibit the binding of the ligand with the receptor may be used.

In addition, a surface-active agent such as CHAPS, Tween 80TM (Kao-Atlas, Japan), digitonin, deoxycholate, etc. and/or various proteins such as bovine serum albumin (BSA), gelatine, etc. may be added to the buffer with an object of decreasing the nonspecific binding. Further, a protease inhibitor such as PMSF, leupeptin, E-64 (manufactured by Peptide Laboratory, Japan), pepstatin, etc. may be added with an object of inhibiting the decomposition of the receptor and the ligand by protease. A labeled ligand in a certain amount (5,000 cpm to 500,000 cpm) is added to 0.01 ml to 10 ml of said receptor solution and, at the same time, 10^{-4} M to 10^{-10} M of a test compound is made copresent. In order to determine the nonspecific binding amount (NSB), a reaction tube to which a great excessive amount of unlabeled test compounds is added is prepared as well.

The reaction is carried out at 0-50°C (preferably at 4-37°C) for 20 minutes to 24 hours (preferably 30 minutes to three hours). After the reaction, it is filtered through a

glass fiber filter, a filter paper, or the like, washed with a suitable amount of the same buffer and the radioactivity retained in the glass fiber filter, etc. is measured by means of a liquid scintillation counter or a gamma-counter.

5 Supposing that the count ($B_0 - \text{NSB}$) obtained by subtracting the nonspecific binding amount (NSB) from the total binding amount (B_0) wherein an antagonizing substance is not present is set at 100%, the test compound in which the specific binding amount ($B - \text{NSB}$) obtained by subtracting the nonspecific binding
10 amount (NSB) from the total binding amount (B) is, for example, less than 50% may be selected as a candidate ligand to the G protein coupled receptor protein of the present invention.

 In conducting the above-mentioned methods ④ to ⑤ for screening the compound which inhibits the binding of the
15 ligand with the G protein coupled receptor protein, the G protein coupled receptor protein-mediated cell stimulating activity (e.g. activities of promoting or activities of inhibiting physiological responses such as liberation of arachidonic acid, liberation of acetylcholine, endocellular
20 Ca^{2+} liberation, endocellular cAMP production, production of inositol phosphate, changes in the cell membrane potential, phosphorylation of endocellular proteins, activation of c-fos, lowering of pH, activation of G protein and cell promulgation, etc.) may be measured by known methods or by the use of
25 commercially available measuring kits. To be more specific, G protein coupled receptor protein-containing cells are at first cultured in a multiwell plate or the like.

 In conducting the screening, it is substituted with a suitable buffer which does not show toxicity to fresh media
30 or cells in advance, incubated for a certain period after adding a test compound, etc. thereto. The resultant cells are extracted or the supernatant liquid is recovered and the resulting product is determined, preferably quantitatively, by each of the methods. When it is difficult to identify the
35 production of the index substance (e.g. arachidonic acid, etc.) which is to be an index for the cell stimulating activity due to the presence of decomposing enzymes contained in the cell,

an assay may be carried out by adding an inhibitor against said decomposing enzyme. With respect to the activities such as an inhibitory action against cAMP production, it may be detected as an inhibitory action against the cAMP production in the cells whose fundamental production has been increased by forskolin or the like.

In conducting a screening by measuring the cell stimulating activity, cells in which a suitable G protein coupled receptor protein is expressed are necessary.

Preferred G protein coupled receptor protein-expressing cells are naturally occurring G protein coupled receptor protein (natural type G protein coupled receptor protein)-containing cell lines or strains (e.g. mouse pancreatic β cell line, MIN6, etc.), the above-mentioned recombinant type G protein coupled receptor protein-expressing cell lines or strains, etc.

Examples of the test compound includes peptides, proteins, non-peptidic compounds, synthesized compounds, fermented products, cell extracts, plant extracts, animal tissue extracts, serum, blood, body fluid, etc. Those compounds may be novel or known.

A kit for screening the compound which inhibits the binding of the ligand with the G protein coupled receptor protein or a salt thereof of the present invention comprises a G protein coupled receptor protein or a peptide fragment thereof, or G protein coupled receptor protein-containing cells or cell membrane fraction thereof.

Examples of the screening kit include as follows:

1. Reagent for Determining Ligand.

① Buffer for Measurement and Buffer for Washing.

The product wherein 0.05% of bovine serum albumin (manufactured by Sigma) is added to Hanks' Balanced Salt Solution (manufactured by Gibco).

This may be sterilized by filtration through a membrane filter with a 0.45 μ m pore size, and stored at 4°C or may be prepared upon use.

② Sample of G Protein Conjugated Receptor Protein.

CHO cells in which a G protein coupled receptor protein is expressed are subcultured at the rate of 5×10^5 cells/well in a 12-well plate and cultured at 37°C with a 5% CO₂ and 95% air atmosphere for two days to prepare the sample.

③ Labeled Ligand.

The ligand which is labeled with commercially available [³H], [¹²⁵I], [¹⁴C], [³⁵S], etc.

The product in a state of an aqueous solution is stored at 4°C or at -20°C and, upon use, diluted to 1 μM with a buffer for the measurement.

④ Standard Ligand Solution.

Ligand is dissolved in PBS containing 0.1% of bovine serum albumin (manufactured by Sigma) to make 1 mM and stored at -20°C.

2. Method of the Measurement.

① CHO cells are cultured in a 12-well tissue culture plate to express G protein coupled receptor proteins. The G protein coupled receptor protein-expressing CHO cells are washed with 1 ml of buffer for the measurement twice. Then 490 μl of buffer for the measurement is added to each well.

② Five μl of a test compound solution of 10^{-3} to 10^{-10} M is added, then 5 μl of a labeled ligand is added and is made to react at room temperature for one hour. For knowing the non-specific binding amount, 5 μl of the ligand of 10^{-3} M is added instead of the test compound.

③ The reaction solution is removed from the well, which is washed with 1 ml of buffer for the measurement three times. The labeled ligand binding with the cells is dissolved in 0.2N NaOH-1% SDS and mixed with 4 ml of a liquid scintillator A (manufactured by Wako Pure Chemical, Japan).

④ Radioactivity is measured using a liquid scintillation counter (manufactured by Beckmann) and PMB (percent of maximum binding) is calculated by the following expression:

$$\text{PMB} = [(B - \text{NSB}) / (B_0 - \text{NSB})] \times 100$$

PMB: Percent of maximum binding
B: Value when a sample is added
NSB: Nonspecific binding
 B_0 : Maximum binding

5 The compound or a salt thereof obtained by the
screening method or by the screening kit is a compound which
inhibits the binding of a ligand with a G protein coupled
receptor protein and, more particularly, it is a compound
having a cell stimulating activity mediated via a G protein
10 coupled receptor or a salt thereof (so-called "G protein
coupled receptor agonist") or a compound having no said
stimulating activity (so-called "G protein coupled receptor
antagonist"). Examples of said compound are peptides,
proteins, non-peptidic compounds, synthesized compounds,
15 fermented products, etc. and the compound may be novel or
known.

Said G protein coupled receptor agonist has the same
physiological action as the ligand to the G protein coupled
receptor protein has and, therefore, it is useful as a safe
20 and less toxic pharmaceutical composition depending upon said
ligand activity.

On the other hand, said G protein coupled receptor
antagonist is capable of inhibiting the physiological activity
of the ligand to the G protein coupled receptor protein and,
25 there fore, it is useful as a safe and less toxic
pharmaceutical composition for inhibiting said ligand
activity.

It is also strongly suggested that agonists and/or
antagonists related to the receptor encoded by pMAH2-17
30 obtained in Example 19 and/or the receptor encoded by phAH2-17
obtained in Example 21 would be useful in therapeutic or
prophylactic treatment of diseases or syndromes in connection
with purine ligand compounds or related analogues. It is
expected that the agonists of the receptor encoded by pMAH2-17
35 and/or of the receptor encoded by phAH2-17 are useful as an
immunomodulator or an antitumor agent, in addition they are

useful in therapeutically or prophylactically treating hypertension, diabetes, cystic fibrosis, etc. It is still expected that the antagonists of the receptor encoded by pMAH2-17 and/or of the receptor encoded by pAH2-17 are useful
5 as hypotensive agents, analgesics, agents for therapeutically or prophylactically treating incontinence of urine, etc. With regard to purinoceptors, the mutation of conserved basic amino acid residues in the 6th or 7th putative transmembrane domain of purinoceptors introduces alteration into the receptor's
10 responses to ATP (J. Biol. Chem., Vol. 270(9), pp. 4185-4188 (1995)). It is suggested that ATP is related to blood pressure control and circular systems via receptors (Circulation Research, Vol. 58(3), pp. 319-330 (1986)) and that ATP and purinoceptors are closely related (Am. Phys. Soc., pp.
15 C577-C606 (1993)).

When the compound or the salt thereof obtained by the screening method or by the screening kit is used as the above-mentioned pharmaceutical composition, a conventional means may be applied therefor. The compound or the salt
20 thereof may be orally, parenterally, by inhalation spray, rectally, or topically administered as pharmaceutical compositions or formulations (e.g. powders, granules, tablets, pills, capsules, injections, syrups, emulsions, elixirs, suspensions, solutions, etc.). For example, it may be used
25 by an oral route as tablets (sugar-coated if necessary), capsules, elixiers, microcapsules, etc. or by a parenteral route as injections such as an aseptic solution or a suspension in water or in other pharmaceutically acceptable liquid. The pharmaceutical compositions or formulations may
30 comprise at least one such compound alone or in admixture with pharmaceutically acceptable carriers, adjuvants, vehicles, excipients and/or diluents. The pharmaceutical compositions can be formulated in accordance with conventional methods. For example, said compound or the salt thereof is mixed in a
35 unit dose form which is required for preparing a generally approved pharmaceutical preparations together with a

physiologically acceptable carriers, flavoring and/or perfuming agents (fragrances), fillers, vehicles, antiseptics, stabilizers, binders, etc. whereupon the preparation can be manufactured. An amount of the effective component in those
5 preparations is to be in such an extent that the suitable dose within an indicated range is achieved.

Examples of the additives which can be admixed in the tablets, capsules, etc. are binders such as gelatin, corn starch, tragacanth and gum arabicum; fillers such as
10 crystalline cellulose; swelling agents such as corn starch, gelatin and alginic acid; lubricants such as magnesium stearate; sweetening agents such as sucrose, lactose and saccharine; preservatives such as parabens and sorbic acid; antioxidants such as ascorbic acid, α -tocopherol and cysteine;
15 fragrances such as peppermint, akamono oil and cherry; disintegrants; buffering agents; etc. Other additives may include mannitol, maltitol, dextran, agar, chitin, chitosan, pectin, collagen, casein, albumin, synthetic or semi-synthetic polymers, glyceride, lactide, etc. When the unit form of the
20 preparation is a capsule, a liquid carrier such as fat/oil may be further added besides the above-mentioned types of materials. The aseptic composition for injection may be formulated by a conventional technique or practice for the preparations such as that the active substance in a vehicle
25 such as water for injection is dissolved or suspended in a naturally occurring plant oil such as sesame oil and palm oil.

Examples of an aqueous liquid for the injection are a physiological saline solution and isotonic solutions containing glucose and other auxiliary agents (e.g. D-sorbitol,
30 D-mannitol, sodium chloride, etc.) wherein a suitable auxiliary solubilizers such as alcohol (e.g. ethanol, etc.), polyalcohol (e.g. propylene glycol, polyethylene glycol, etc.), nonionic surface-active agent (e.g. Polysorbate 80TM, HCO-50, etc.), etc. may be jointly used. In the case of the oily liquid,
35 sesame oil, soybean oil, etc. may be exemplified wherein benzyl benzoate, benzyl alcohol, etc. may be jointly used as auxiliary solubilizers.

In addition, buffers (e.g. phosphate buffer, sodium acetate buffer, etc.), analgesic agents (e.g. benzalkonium chloride, procaine hydrochloride, etc.), stabilizers (e.g. human serum albumin, polyethylene glycol, etc.), stabilizers (e.g. benzyl alcohol, phenol, etc.), antioxidants, etc. may be compounded therewith too. The prepared injection solution is filled in suitable ampoules. The formulation prepared as such is safe and less toxic and, therefore, it can be administered to warm-blooded mammals such as rats, rabbits, sheep, swines, cattle, cats, dogs, monkeys, human being, etc.

Dose levels of said compound or the salt thereof may vary depending upon the symptom. Specific dose levels for any particular patient will be employed depending upon a variety of factors including the activity of specific compounds employed, the age, body weight, general health, sex, diet, time of administration, route of administration, rate of excretion, drug combination, and the severity of the particular disease undergoing therapy. In the case of oral administration, it is usually about 0.1-100 mg, preferably about 1.0-50 mg or, more preferably, about 1.0-20 mg per day for adults (as 60 kg). When it is administered parenterally, its dose at a time may vary depending upon the object to be administered, organs to be administered, symptoms, administering methods, etc. The term "parenteral" as used herein includes subcutaneous injections, intravenous, intramuscular, intraperitoneal injections, or infusion techniques. In the case of injections, it is usually convenient to give by an intravenous route in an amount of about 0.01-30 mg, preferably about 0.1-20 mg or, more preferably, about 0.1-10 mg per day to adults (as 60 kg). In the case of other animals, the dose calculated for 60 kg may be administered as well.

(6) Manufacture of Antibody or Antiserum against the G Protein Coupled Receptor Protein of the Present Invention, Its Peptide Fragment or Its Salt.

Antibodies (e.g. polyclonal antibody and monoclonal antibody) and antisera against the G protein coupled receptor

protein or salt thereof of the present invention or against the peptide fragment of the G protein coupled receptor protein or salt thereof of the present invention may be manufactured by antibody or antiserum-manufacturing methods per se known to those of skill in the art or methods similar thereto, using the G protein coupled receptor protein or its salt of the present invention or the peptide fragment of the G protein coupled receptor protein or its salt of the present invention. For example, monoclonal antibodies can be manufactured by the method as given below.

[Preparation of Monoclonal Antibody]

(a) Preparation of Monoclonal Antibody-Producing Cells.

The G protein coupled receptor protein of the present invention or its salt or the peptide fragment of the G protein coupled receptor protein of the present invention or its salt (hereinafter, may be abbreviated as the "G protein coupled receptor protein") is administered to warm-blooded animals either solely or together with carriers or diluents to the site where the production of antibody is possible by the administration. In order to potentiate the antibody productivity upon the administration, complete Freund's adjuvants or incomplete Freund's adjuvants may be administered. The administration is usually carried out once every two to six weeks and two to ten times in total. Examples of the applicable warm-blooded animals are monkeys, rabbits, dogs, guinea pigs, mice, rats, sheep, goats and chickens and the use of mice and rats is preferred.

In the preparation of the cells which produce monoclonal antibodies, an animal wherein the antibody titer is noted is selected from warm-blooded animals (e.g. mice) immunized with antigens, then spleen or lymph node is collected after two to five days from the final immunization and antibody-producing cells contained therein are fused with myeloma cells to give monoclonal antibody-producing hybridomas. Measurement of the antibody titer in antisera may, for example, be carried out by reacting a labeled

G protein coupled receptor protein (which will be mentioned later) with the antiserum followed by measuring the binding activity of the labeling agent with the antibody.

The operation for fusing may be carried out, for example, by a method of Koehler and Milstein (Nature, 256, 495, 1975). Examples of the fusion accelerator are polyethylene glycol (PEG), Sendai virus, etc. and the use of PEG is preferred.

Examples of the myeloma cells are NS-1, P3U1, SP2/0, AP-1, etc. and the use of P3U1 is preferred. The preferred fusion ratio of the numbers of antibody-producing cells used (spleen cells) to the numbers of myeloma cells is within a range of about 1:1 to 20:1. When PEG (preferably, PEG 1000 to PEG 6000) is added in a concentration of about 10-80% followed by incubating at 20-40°C (preferably, at 30-37°C) for one to ten minutes, an efficient cell fusion can be carried out.

Various methods may be applied for screening a hybridoma which produces anti-G protein coupled receptor antibody. For example, a supernatant liquid of hybridoma culture is added to a solid phase (e.g. microplate) to which the G protein coupled receptor protein antigen is adsorbed either directly or with a carrier, then anti-immunoglobulin antibody (anti-mouse immunoglobulin antibody is used when the cells used for the cell fusion are those of mouse) which is labeled with a radioactive substance, an enzyme or the like, or protein A is added thereto and then anti-G protein coupled receptor monoclonal antibodies bound on the solid phase are detected; or a supernatant liquid of the hybridoma culture is added to the solid phase to which anti-immunoglobulin or protein A is adsorbed, then the G protein coupled receptor labeled with a radioactive substance or an enzyme is added and anti-G protein coupled receptor monoclonal antibodies bonded with the solid phase is detected.

Selection and cloning of the anti-G protein coupled receptor monoclonal antibody-producing hybridoma may be carried out by methods per se known to those of skill in the art or methods similar thereto. Usually, it is carried out in a

medium for animal cells, containing HAT (hypoxanthine, aminopterin and thymidine). With respect to a medium for the selection, for the cloning and for the growth, any medium may be used so far as hybridoma is able to grow therein. Examples of the medium are an RPMI 1640 medium (Dainippon Pharmaceutical Co., Ltd., Japan) containing 1-20% (preferably 10-20%) of fetal calf serum (FCS), a GIT medium (Wako Pure Chemical, Japan) containing 1-20% of fetal calf serum and a serum-free medium for hybridoma culturing (SFM-101; Nissui Seiyaku, Japan).

The culturing temperature is usually 20-40°C and, preferably, about 37°C. The culturing time is usually from five days to three weeks and, preferably, one to two weeks. The culturing is usually carried out in 5% carbon dioxide gas. The antibody titer of the supernatant liquid of the hybridoma culture may be measured by the same manner as in the above-mentioned measurement of the antibody titer of the anti-G protein coupled receptor in the antiserum.

The cloning can be usually carried out by methods known per se such as techniques in semi-solid agar and limiting dilution. The cloned hybridoma is preferably cultured in modern serum-free culture media to obtain optimal amounts of antibody in supernatants. The target monoclonal antibody is also preferably obtained from ascitic fluid derived from a mouse, etc. injected intraperitoneally with live hybridoma cells.

(b) Purification of the Monoclonal Antibody.

Like in the separation/purification of conventional polyclonal antibodies, the separation/purification of the anti-G protein coupled receptor monoclonal antibody may be carried out by methods for separating/purifying immunoglobulin (such as salting-out, precipitation with an alcohol, isoelectric precipitation, electrophoresis, adsorption/deadsorption using ion exchangers such as DEAE, ultracentrifugation, gel filtration, specific purifying methods in which only an antibody is collected by treatment with an active adsorbent (such as an antigen-binding solid

phase, protein A or protein G) and the bond is dissociated whereupon the antibody is obtained.

The G protein coupled receptor antibody of the present invention which is manufactured by the aforementioned method (a) or (b) is capable of specifically recognizing G protein coupled receptors and, accordingly, it can be used for a quantitative determination of the G protein coupled receptor in test liquid samples and particularly for a quantitative determination by sandwich immunoassays.

Thus, the present invention provides, for example, the following methods:

- (i) a quantitative determination of a G protein coupled receptor in a test liquid sample, which comprises
 - (a) competitively reacting the test liquid sample and a labeled G protein coupled receptor with an antibody which reacts with the G protein coupled receptor of the present invention, and
 - (b) measuring the ratio of the labeled G protein coupled receptor binding with said antibody; and
- (ii) a quantitative determination of a G protein coupled receptor in a test liquid sample, which comprises
 - (a) reacting the test liquid sample with an antibody immobilized on an insoluble carrier and a labeled antibody simultaneously or continuously, and
 - (b) measuring the activity of the labeling agent on the insoluble carrier

wherein one antibody is capable of recognizing the N-terminal region of the G protein coupled receptor while another antibody is capable of recognizing the C-terminal region of the G protein coupled receptor.

When the monoclonal antibody of the present invention recognizing a G protein coupled receptor (hereinafter, may be referred to as "anti-G protein coupled receptor antibody") is used, G protein coupled receptors can be measured and, moreover, can be detected by means of a tissue staining, etc. as well. For such an object, antibody molecules per se may be used or $F(ab')_2$, Fab' or Fab fractions of the antibody

molecule may be used too. There is no particular limitation for the measuring method using the antibody of the present invention and any measuring method may be used so far as it relates to a method in which the amount of antibody, antigen or antibody-antigen complex, depending on or corresponding to the amount of antigen (e.g. the amount of G protein coupled receptor, etc.) in the liquid sample to be measured, is detected by a chemical or a physical means and then calculated using a standard curve prepared by a standard solution containing the known amount of antigen. For example, nephrometry, competitive method, immunometric method and sandwich method are suitably used and, in terms of sensitivity and specificity, the sandwich method which will be described herein later is particularly preferred.

Examples of the labeling agent used in the measuring method using the labeling substance are radioisotopes, enzymes, fluorescent substances, luminescent substances, colloids, magnetic substances, etc. Examples of the radioisotope are [^{125}I], [^{131}I], [^3H] and [^{14}C]; preferred examples of the enzyme are those which are stable and with big specific activity, such as β -galactosidase, β -glucosidase, alkali phosphatase, peroxidase and malate dehydrogenase; examples of the fluorescent substance are fluorescamine, fluorescein isothiocyanate, etc.; and examples of the luminescent substance are luminol, luminol derivatives, luciferin, lucigenin, etc. Further, a biotin-avidin system may also be used for binding an antibody or antigen with a labeling agent.

In an insolubilization (immobilization) of antigens or antibodies, a physical adsorption may be used or a chemical binding which is usually used for insolubilization or immobilization of proteins or enzymes may be used as well. Examples of the carrier are insoluble polysaccharides such as agarose, dextran and cellulose; synthetic resins such as polystyrene, polyacrylamide and silicone; glass; etc.

In a sandwich (or two-site) method, the test liquid is made to react with an insolubilized anti-G protein coupled

receptor antibody (the first reaction), then it is made to react with a labeled anti-G protein coupled receptor antibody (the second reaction) and the activity of the labeling agent on the insoluble carrier is measured whereupon the amount of the G protein coupled receptor in the test liquid can be determined. The first reaction and the second reaction may be conducted reversely or simultaneously or they may be conducted with an interval. The type of the labeling agent and the method of insolubilization (immobilization) may be the same as those mentioned already herein. In the immunoassay by means of a sandwich method, it is not always necessary that the antibody used for the labeled antibody and the antibody for the solid phase is one type or one species but, with an object of improving the measuring sensitivity, etc., a mixture of two or more antibodies may be used too.

In the method of measuring G protein coupled receptors by the sandwich method of the present invention, the preferred anti-G protein coupled receptor antibodies used for the first and the second reactions are antibodies wherein their sites binding to the G protein coupled receptors are different each other. Thus, the antibodies used in the first and the second reactions are those wherein, when the antibody used in the second reaction recognizes the C-terminal region of the G protein coupled receptor, then the antibody recognizing the site other than C-terminal regions, e.g. recognizing the N-terminal region, is preferably used in the first reaction.

The anti-G protein coupled receptor antibody of the present invention may be used in a measuring system other than the sandwich method such as a competitive method, an immunometric method and a nephrometry. In a competitive method, an antigen in the test solution and a labeled antigen are made to react with an antibody in a competitive manner, then an unreacted labeled antigen (F) and a labeled antigen binding with an antibody (B) are separated (i.e. B/F separation) and the labeled amount of any of B and F is measured whereupon the amount of the antigen in the test

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solution is determined. With respect to a method for such a reaction, there are a liquid phase method in which a soluble antibody is used as the antibody and the B/F separation is conducted by polyethylene glycol, a second antibody to the above-mentioned antibody, etc.; and a solid phase method in which an immobilized antibody is used as the first antibody or a soluble antibody is used as the first antibody while an immobilized antibody is used as the second antibody.

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In an immunometric method, an antigen in the test solution and an immobilized antigen are subjected to a competitive reaction with a certain amount of a labeled antibody followed by separating into solid and liquid phases; or the antigen in the test solution and an excess amount of labeled antibody are made to react, then a immobilized antigen is added to bind an unreacted labeled antibody with the solid phase and separated into solid and liquid phases. After that, the labeled amount of any of the phases is measured to determine the antigen amount in the test solution.

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In a nephrometry, the amount of insoluble sediment which is produced as a result of the antigen-antibody reaction in a gel or in a solution is measured. Even when the antigen amount in the test solution is small and only a small amount of the sediment is obtained, a laser nephrometry wherein scattering of laser is utilized can be suitably used.

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In applying each of those immunological measuring methods (immunoassays) to the measuring method of the present invention, it is not necessary to set up any special condition, operation, etc. therefor. A measuring system (assay system) for G protein coupled receptor may be constructed taking the technical consideration of the persons skilled in the art into consideration in the conventional conditions and operations for each of the methods. With details of those conventional technical means, a variety of reviews, reference books, etc. may be referred to. They are, for example, Hiroshi Irie (ed): "Radioimmunoassay" (Kodansha, Japan, 1974); Hiroshi Irie (ed): "Radioimmunoassay; Second Series" (Kodansha, Japan, 1979); Eiji Ishikwa et al. (ed): "Enzyme Immunoassay" (Igaku Shoin,

Japan, 1978); Eiji Ishikawa et al. (ed): "Enzyme Immunoassay" (Second Edition) (Igaku Shoin, Japan, 1982); Eiji Ishikawa et al. (ed): "Enzyme Immunoassay" (Third Edition) (Igaku Shoin, Japan, 1987); "Methods in Enzymology" Vol. 70 (Immunochemical Techniques (Part A)); ibid. Vol. 73 (Immunochemical Techniques (Part B)); ibid. Vol. 74 (Immunochemical Techniques (Part C)); ibid. Vol. 84 (Immunochemical Techniques (Part D: Selected Immunoassays)); ibid. Vol. 92 (Immunochemical Techniques (Part E: Monoclonal Antibodies and General Immunoassay Methods)); ibid. Vol. 121 (Immunochemical Techniques (Part I: Hybridoma Technology and Monoclonal Antibodies)) (Academic Press); etc.

(7) Preparation of Animals Having the G Protein Coupled Receptor Protein-Encoding DNA of the Present Invention.

It is possible to prepare transgenic animals expressing G protein coupled receptors using G protein coupled receptor protein-encoding DNA. Examples of the animals are warm-blooded mammals such as rats, rabbit, sheep, swines, cattle, cats, dogs and monkeys.

In transferring the G protein coupled receptor protein-encoding DNA to the aimed animal, it is generally advantageous that said DNA is used by ligating with a site at the downstream of a promoter which is capable of expressing in animal cells. For example, when G protein coupled receptor protein DNA is to be transferred to a rabbit, a gene construct ligated with a site at the downstream of various promoters which are capable of expressing the G protein coupled receptor protein DNA derived from an animal compatible to the animal in animal host cells is subjected to a microinjection to the fertilized ovum (oosperm) of the aimed animal (e.g. fertilized ovum (embryo) of rabbit) whereupon the transgenic animal which produces the G protein coupled receptor protein in a high amount can be prepared.

Examples of the promoters used are promoters derived from virus and ubiquitous expression promoters such as metallothionein promoters may be used but, preferably,

enolase gene promoters and NGF gene promoters capable of specifically expressing in brain are used.

Transfer of the G protein coupled receptor protein DNA at a fertilized ovum cell stage is secured in order that the DNA can be present in all of embryonal cells and body somatic cells of an aimed animal. The fact that the G protein coupled receptor protein is present in the fertilized ovum cells of the produced transgenic animal after the DNA transfer means that all progeny of the produced transgenic animal have the G protein coupled receptor protein in all of their embryonal cells and somatic cells. Descendants (offsprings) of the animal of this type which inherited the gene have the G protein coupled receptor protein in all of their embryonal cells and somatic cells.

The transgenic animal to which the G protein coupled receptor protein DNA is transferred can be subjected to a mating and a breeding for generations under a common breeding circumstance as the animal holding said DNA after confirming that the gene can be stably retained. Moreover, male and female animals having the desired DNA are mated to give a homozygote having the transduced gene in both homologous chromosomes and then those male and female animals are mated whereby it is possible to breed for generations so that all descendants have said DNA.

The animal to which the G protein coupled receptor protein DNA is transferred highly expresses the G protein coupled receptor protein and, accordingly, it is useful as the animal for screening for an agonist or an antagonist to said G protein coupled receptor protein.

The DNA-transferred animal can be used as a cell source for a tissue culture. For example, DNA or RNA in the tissue of the DNA-transferred mouse is directly analyzed or protein tissues expressed by gene are analyzed whereupon the G protein coupled receptor protein can be analyzed.

Cells of the G protein coupled receptor protein-containing tissue are cultured by standard tissue culture techniques whereupon it is possible to study the function of the cells

which are usually difficult to culture (e.g. those derived from brain and peripheral tissues) using the resulting culture. By using said cells, it is also possible to select the pharmaceuticals which can potentiate, for example, the functions of various tissues. Moreover, if a cell strain with a high expression is available, it is possible to separate and purify G protein coupled receptor proteins therefrom.

As such, the amount of G protein coupled receptor proteins can now be determined with a high precision using the anti-G protein coupled receptor antibody of the present invention.

(8) Antisense Oligonucleotides Capable of Inhibiting Replication of G Protein Coupled Receptor Protein Gene

In another aspect of the present invention, antisense oligonucleotides (nucleic acids) capable of inhibiting the replication or expression of G protein coupled receptor protein gene may be designed and synthesized based on information on the nucleotide sequences of cloned and determined G protein coupled receptor protein-encoding DNAs. Such an antisense oligonucleotide (nucleic acid) is capable of hybridizing with RNA of G protein coupled receptor protein genes to inhibit the synthesis or function of said RNA or of modulating the expression of a G protein coupled receptor protein gene via interaction with G protein coupled receptor protein-related RNA. Oligonucleotides complementary to, and specifically hybridizable with, selected sequences of G protein coupled receptor protein-related RNA are useful in controlling or modulating the expression of a G protein coupled receptor protein gene in vitro and in vivo, and in treating or diagnosing disease states of suspected animals. The term "corresponding" means homologous to or complementary to a particular sequence of the nucleotide sequence or nucleic acid including the gene. As between nucleotides (nucleic acids) and peptides (proteins), "corresponding" usually refers to amino acids of a peptide (protein) in an order derived from the sequence of a nucleotides (nucleic acids) or its complement.

The G protein coupled receptor protein gene 5' end hairpin loop, 5' end 6-base-pair repeats, 5' end untranslated region, polypeptide translation initiation codon, protein coding region, ORF translation initiation codon, 3' untranslated region, 3' end palindrome region, and 3' end hairpin loop may be selected as preferred targets though any region may be a target among G protein coupled receptor protein genes. The relationship between the target and oligonucleotides complementary to at least a portion of the target, specifically hybridizable with the target, is denoted as "antisense". The antisense oligonucleotides may be polydeoxynucleotides containing 2-deoxy-D-ribose, polyribonucleotides containing D-ribose, any other type of polynucleotide which is an N-glycoside of a purine or pyrimidine base, or other polymers containing nonnucleotide backbones (e.g., protein nucleic acids and synthetic sequence-specific nucleic acid polymers commercially available) or nonstandard linkages, providing that the polymers contain nucleotides in a configuration which allows for base pairing and base stacking such as is found in DNA and RNA. They may include double- and single-stranded DNA, as well as double- and single-stranded RNA and DNA:RNA hybrids, and also include, as well as unmodified forms of the polynucleotide or oligonucleotide, known types of modifications, for example, labels which are known to those skilled in the art, "caps", methylation, substitution of one or more of the naturally occurring nucleotides with analogue, internucleotide modifications such as, for example, those with uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoramidates, carbamates, etc.) and with charged linkages or sulfur-containing linkages (e.g., phosphorothioates, phosphorodithioates, etc.), those containing pendant moieties, such as, for example, proteins (including nucleases, nuclease inhibitors, toxins, antibodies, signal peptides, poly-L-lysine, etc.) and saccharides (e.g., monosaccharides, etc.), those with intercalators (e.g., acridine, psoralen, etc.), those containing chelators (e.g., metals, radioactive metals, boron, oxidative metals, etc.),

those containing alkylators, those with modified linkages (e.g., alpha anomeric nucleic acids, etc.). The terms "nucleoside", "nucleotide" and "nucleic acid" will include those moieties which contain not only the known purine and pyrimidine bases, but also other heterocyclic bases which have been modified. Such modifications include methylated purines and pyrimidines, acylated purines and pyrimidines, or other heterocycles. Modified nucleosides or nucleotides will also include modifications on the sugar moiety, e.g., wherein one or more of the hydroxyl groups are replaced with halogen, aliphatic groups, or are functionalized as ethers, amines, or the like.

The antisense nucleic acid of the present invention is RNA, DNA or a modified nucleic acid. Examples of modified nucleic acid are, but not limited to, degradation-resistant sulfurized and thiophosphate derivatives of nucleic acids, and poly- or oligonucleoside amides. Preferred design modifications of the antisense nucleic acids of the present invention are modifications that are designed to:

- (1) increase the intracellular stability of the nucleic acid;
- (2) increase the cellular permeability of the nucleic acid;
- (3) increase the affinity of the nucleic acid for the target sense strand; or
- (4) decrease the toxicity (if any) of the nucleic acid.

Many such modifications are known to those skilled in the art, as described in J. Kawakami et al., Pharm Tech Japan, Vol. 8, pp.247, 1992; Vol. 8, pp.395, 1992; S. T. Crooke et al. ed., Antisense Research and Applications, CRC Press, 1993; etc. The nucleic acids may contain altered or modified sugars, bases or linkages, be delivered in specialized systems such as liposomes, microspheres or by gene therapy, or may have attached moieties. Such attached moieties include polycationic moieties such as polylysine that act as charge neutralizers of the phosphate backbone, or hydrophobic moieties such as lipids (e.g., phospholipids, cholesterol, etc.) that enhance interaction with cell membranes or increase uptake of the nucleic acid. Preferred lipids that may be attached are

cholesterols or derivatives thereof (e.g., cholesteryl
chloroformate, cholic acid, etc.). The moieties may be
attached at the 3' or 5' ends of the nucleic acids, and also
may be attached through a base, sugar, or internucleoside
5 linkage. Other moieties may be capping groups specifically
placed at the 3' or 5' ends of the nucleic acids to prevent
degradation by nuclease such as exonuclease, RNase, etc. Such
capping groups include, but are not limited to, hydroxyl
protecting groups known to those skilled in the art, including
10 glycols such as polyethylene glycols, tetraethylene glycol and
the like.

The inhibitory activity of antisense nucleic acids
can be examined using the transformant (or transfectant) of
the present invention, the in vitro and in vivo gene
15 expression system of the present invention, or the in vitro
and in vivo translation system of G protein coupled receptor
proteins. The nucleic acid can be placed in the cell through
any number of ways known per se.

In the specification and drawings of the present
20 application, the abbreviations used for bases (nucleotides),
amino acids and so forth are those recommended by the IUPAC-IUB
Commission on Biochemical Nomenclature or those conventionally
used in the art. Examples thereof are given below.
Amino acids for which optical isomerism is possible are, unless
25 otherwise specified, in the L form.

DNA : Deoxyribonucleic acid

cdNA: Complementary deoxyribonucleic acid

A : Adenine

T : Thymine

30 G : Guanine

C : Cytosine

RNA : Ribonucleic acid

mRNA : Messenger ribonucleic acid

dATP: Deoxyadenosine triphosphate

35 dTTP: Deoxythymidine triphosphate

dGTP: Deoxyguanosine triphosphate
dCTP: Deoxycytidine triphosphate
ATP : Adenosine triphosphate
EDTA: Ethylenediamine tetraacetic acid

5 SDS : Sodium dodecyl sulfate

EIA: Enzyme Immunoassay

G, Gly: Glycine (or Glycyl)

A, Ala: Alanine (or Alanyl)

V, Val: Valine (or Valyl)

10 L, Leu: Leucine (or Leucyl)

I, Ile: Isoleucine (or Isoleucyl)

S, Ser: Serine (or Seryl)

T, Thr: Threonine (or Threonyl)

C, Cys: Cysteine (or Cysteinyl)

15 M, Met: Methionine (or Methionyl)

E, Glu: Glutamic acid (or Glutamyl)

D, Asp: Aspartic acid (or Aspartyl)

K, Lys: Lysine (or Lysyl)

R, Arg: Arginine (or Arginyl)

20 H, His: Histidine (or Histidyl)

F, Phe: Pheylalanine (or Pheylalanyl)

Y, Tyr: Tyrossine (or Tyrosyl)

W, Trp: Tryptophan (or Tryptophanyl)

P, Pro: Proline (or Prolyl)

25 N, Asn: Asparagine (or Asparaginyl)

Q, Gln: Glutamine (or Glutaminyl)

NVal: Norvaline (or Norvalyl)

pGlu: Pyroglutamic acid (or Pyroglutamyl)

Blc: γ -Butyrolacton- γ -carbonyl

30 Kpc: 2-Ketopiperidiny1-6-carbonyl

Otc: 3-Oxoperhydro-1,4-thiazin-5-carbonyl

Me: Methyl

Et: Ethyl

Bu: Butyl

35 Ph: Phenyl

TC: Thiazolidiny1-4(R)-carboxamide

The transformant *Escherichia coli*, designated INV α F'/p19P2, which is obtained in the Example 3 mentioned herein below, is on deposit under the terms of the Budapest Treaty from August 9, 1994, with the National Institute of Bioscience and Human-Technology (NIBH), Agency of Industrial Science and Technology, Ministry of International Trade and Industry, Japan and has been assigned the Accession Number FERM BP-4776. It is also on deposit from August 22, 1994 with the Institute for Fermentation, Osaka, Japan (IFO) and has been assigned the Accession Number IFO 15739.

The transformant *Escherichia coli*, designated INV α F'/pG3-2, which is obtained in the Example 4 mentioned herein below, is on deposit under the terms of the Budapest Treaty from August 9, 1994, with NIBH and has been assigned the Accession Number FERM BP-4775. It is also on deposit from August 22, 1994 with IFO and has been assigned the Accession Number IFO 15740.

The transformant *Escherichia coli*, designated INV α F'/p63A2, which is obtained in the Example 5 mentioned herein below, is on deposit under the terms of the Budapest Treaty from August 9, 1994, with NIBH and has been assigned the Accession Number FERM BP-4777. It is also on deposit from August 22, 1994 with IFO and has been assigned the Accession Number IFO 15738.

The transformant *Escherichia coli*, designated JM109/phGR3, which is obtained in the Example 6 mentioned herein below, is on deposit under the terms of the Budapest Treaty from September 27, 1994, with NIBH and has been assigned the Accession Number FERM BP-4807. It is also on deposit from September 22, 1994 with IFO and has been assigned the Accession Number IFO 15748.

The transformant *Escherichia coli*, designated JM109/p3H2-17, which is obtained in the Example 7 mentioned herein below, is on deposit under the terms of the Budapest Treaty from September 27, 1994, with NIBH and has been assigned the Accession Number FERM BP-4806. It is also on deposit from September 22, 1994 with IFO and has been assigned the Accession

Number IFO 15747.

The transformant *Escherichia coli*, designated JM109/p3H2-34, which is obtained in the Example 8 mentioned herein below, is on deposit under the terms of the Budapest Treaty from October 12, 1994, with NIBH and has been assigned the Accession Number FERM BP-4828. It is also on deposit from October 12, 1994 with IFO and has been assigned the Accession Number IFO 15749.

The transformant *Escherichia coli*, designated JM109/pMD4, which is obtained in the Example 9 mentioned herein below, is on deposit under the terms of the Budapest Treaty from November 11, 1994, with NIBH and has been assigned the Accession Number FERM BP-4888. It is also on deposit from November 17, 1994 with IFO and has been assigned the Accession Number IFO 15765.

The transformant *Escherichia coli*, designated JM109/pMGR20, which is obtained in the Example 10 mentioned herein below, is on deposit under the terms of the Budapest Treaty from December 15, 1994, with NIBH and has been assigned the Accession Number FERM BP-4937. It is also on deposit from December 14, 1994 with IFO and has been assigned the Accession Number IFO 15773.

The transformant *Escherichia coli*, designated JM109/pMJ10, which is obtained in the Example 12 mentioned herein below, is on deposit under the terms of the Budapest Treaty from December 15, 1994, with NIBH and has been assigned the Accession Number FERM BP-4936. It is also on deposit from December 16, 1994 with IFO and has been assigned the Accession Number IFO 15784.

The transformant *Escherichia coli*, designated JM109/pMH28, which is obtained in the Example 14 mentioned herein below, is on deposit under the terms of the Budapest Treaty from January 13, 1995, with NIBH and has been assigned the Accession Number FERM BP-4970. It is also on deposit from January 20, 1995 with IFO and has been assigned the Accession Number IFO 15791.

The transformant *Escherichia coli*, designated

JM109/pMN7, which is obtained in the Example 16 mentioned herein below, is on deposit under the terms of the Budapest Treaty from February 22, 1995, with NIBH and has been assigned the Accession Number FERM BP-5011. It is also on deposit from February 27, 1995 with IFO and has been assigned the Accession Number IFO 15803.

The transformant *Escherichia coli*, designated JM109/p5S38, which is obtained in the Example 17 mentioned herein below, is on deposit under the terms of the Budapest Treaty from October 27, 1994, with NIBH and has been assigned the Accession Number FERM BP-4856. It is also on deposit from October 25, 1994 with IFO and has been assigned the Accession Number IFO 15754.

The transformant *Escherichia coli*, designated JM109/pMAH2-17, which is obtained in the Example 19 mentioned herein below, is on deposit under the terms of the Budapest Treaty from April 7, 1995, with NIBH and has been assigned the Accession Number FERM BP-5073. It is also on deposit from March 31, 1995 with IFO and has been assigned the Accession Number IFO 15813.

The transformant *Escherichia coli*, designated JM109/pMN128, which is obtained in the Example 20 mentioned herein below, is on deposit under the terms of the Budapest Treaty from March 17, 1995, with NIBH and has been assigned the Accession Number FERM BP-5039. It is also on deposit from March 22, 1995 with IFO and has been assigned the Accession Number IFO 15810.

The transformant *Escherichia coli*, designated JM109/pAH2-17, which is obtained in the Example 21 mentioned herein below, is on deposit under the terms of the Budapest Treaty from July 20, 1995, with NIBH and has been assigned the Accession Number FERM BP-5168. It is also on deposit from July 14, 1995 with IFO and has been assigned the Accession Number IFO 15856.

Each SEQ ID NO set forth in the SEQUENCE LISTING of the specification refers to the following sequence:

[SEQ ID NO: 24] is a partial amino acid sequence of the human pituitary gland-derived G protein coupled receptor protein encoded by the human pituitary gland-derived G protein coupled receptor protein cDNA fragment included in p19P2,

5 [SEQ ID NO: 25] is a partial amino acid sequence of the human pituitary gland-derived G protein coupled receptor protein encoded by the human pituitary gland-derived G protein coupled receptor protein cDNA fragment included in p19P2,

10 [SEQ ID NO: 26] is an entire amino acid sequence of the human pituitary gland-derived G protein coupled receptor protein encoded by the human pituitary gland-derived G protein coupled receptor protein cDNA fragment included in phGR3,

15 [SEQ ID NO: 27] is a partial amino acid sequence of the mouse pancreatic β -cell line, MIN6-derived G protein coupled receptor protein encoded by the mouse pancreatic β -cell line, MIN6-derived G protein coupled receptor protein cDNA fragment having a nucleotide sequence (SEQ ID NO: 32), derived based upon the nucleotide sequences of the mouse pancreatic β -cell line, MIN6-derived G protein coupled receptor protein cDNA fragments each included in pG3-2 and pG1-10,

20 [SEQ ID NO: 28] is a partial amino acid sequence of the mouse pancreatic β -cell line, MIN6-derived G protein coupled receptor protein encoded by p5S38,

25 [SEQ ID NO: 29] is a nucleotide sequence of the human pituitary gland-derived G protein coupled receptor protein cDNA fragment included in p19P2,

[SEQ ID NO: 30] is a nucleotide sequence of the human pituitary gland-derived G protein coupled receptor protein cDNA fragment included in p19P2,

30 [SEQ ID NO: 31] is an entire nucleotide sequence of the human pituitary gland-derived G protein coupled receptor protein cDNA included in phGR3,

35 [SEQ ID NO: 32] is a nucleotide sequence of the mouse pancreatic β -cell line, MIN6-derived G protein coupled receptor protein cDNA, derived based upon the nucleotide sequences of the mouse pancreatic β -cell line, MIN6-derived G protein coupled receptor protein cDNA fragments each

included in pG3-2 and pG1-10,

[SEQ ID NO: 33] is a nucleotide sequence of the mouse pancreatic β -cell line, MIN6-derived G protein cDNA included in p5S38,

5 [SEQ ID NO: 34] is a partial amino acid sequence of the human amygdaloid nucleus-derived G protein coupled receptor protein encoded by the cDNA fragment included in p63A2,

[SEQ ID NO: 35] is a partial amino acid sequence of the human amygdaloid nucleus-derived G protein coupled receptor protein encoded by the cDNA fragment included in p63A2,

10 [SEQ ID NO: 36] is a nucleotide sequence of the human amygdaloid nucleus-derived G protein coupled receptor protein cDNA fragment included in p63A2,

[SEQ ID NO: 37] is a nucleotide sequence of the human amygdaloid nucleus-derived G protein coupled receptor protein cDNA fragment included in p63A2,

15 [SEQ ID NO: 38] is a partial amino acid sequence encoded by the mouse pancreatic β -cell line, MIN6-derived G protein coupled receptor protein cDNA included in p3H2-17,

20 [SEQ ID NO: 39] is a full-length amino acid sequence encoded by the open reading frame of the mouse pancreatic β -cell line, MIN6-derived G protein coupled receptor protein cDNA included in pMAH2-17,

[SEQ ID NO: 40] is a nucleotide sequence of the mouse pancreatic β -cell line, MIN6-derived G protein coupled receptor protein cDNA included in p3H2-17,

[SEQ ID NO: 41] is a nucleotide sequence of the mouse pancreatic β -cell line, MIN6-derived G protein coupled receptor protein cDNA included in pMAH2-17,

30 [SEQ ID NO: 42] is a partial amino acid sequence encoded by the mouse pancreatic β -cell line, MIN6-derived G protein coupled receptor protein cDNA included in p3H2-34,

[SEQ ID NO: 43] is a nucleotide sequence of the mouse pancreatic β -cell line, MIN6-derived G protein coupled receptor protein cDNA fragment included in p3H2-34,

35 [SEQ ID NO: 44] is a partial amino acid sequence encoded by the rabbit gastropyloric part smooth muscle-derived G

protein coupled receptor protein cDNA included in pMD4,
[SEQ ID NO: 45] is a nucleotide sequence of the rabbit
gastropyrolic part smooth muscle-derived G protein coupled
receptor protein cDNA fragment included in pMD4,
5 [SEQ ID NO: 46] is an entire amino acid sequence
encoded by the mouse pancreatic β -cell line, MIN6-derived G
protein coupled receptor protein cDNA included in pMGR20,
[SEQ ID NO: 47] is a nucleotide sequence of the mouse
pancreatic β -cell line, MIN6-derived G protein coupled
10 receptor protein cDNA included in pMGR20,
[SEQ ID NO: 48] is a partial amino acid sequence encoded
by the rabbit gastropyrolic part smooth muscle-derived G
protein coupled receptor protein cDNA included in pMJ10,
[SEQ ID NO: 49] is a nucleotide sequence of the rabbit
15 gastropyrolic part smooth muscle-derived G protein coupled
receptor protein cDNA fragment included in pMJ10,
[SEQ ID NO: 50] is a partial amino acid sequence encoded
by the rabbit gastropyrolic part smooth muscle-derived G
protein coupled receptor protein cDNA included in pMH28,
20 [SEQ ID NO: 51] is a nucleotide sequence of the rabbit
gastropyrolic part smooth muscle-derived G protein coupled
receptor protein cDNA fragment included in pMH28,
[SEQ ID NO: 52] is a partial amino acid sequence encoded
by the rabbit gastropyrolic part smooth muscle-derived G
25 protein coupled receptor protein cDNA included in pMN7,
[SEQ ID NO: 53] is a nucleotide sequence of the rabbit
gastropyrolic part smooth muscle-derived G protein coupled
receptor protein cDNA fragment included in pMN7,
[SEQ ID NO: 54] is a partial amino acid sequence encoded
30 by the rabbit gastropyrolic part smooth muscle-derived G
protein coupled receptor protein cDNA included in pMN128,
[SEQ ID NO: 55] is a nucleotide sequence of the rabbit
gastropyrolic part smooth muscle-derived G protein coupled
receptor protein cDNA fragment included in pMN128,
35 [SEQ ID NO: 56] is a full-length amino acid sequence of the
human-derived G protein coupled receptor protein encoded
by the human-derived G protein coupled receptor protein cDNA

included in pAH2-17, and
[SEQ ID NO: 57] is a nucleotide sequence of the human-derived
G protein coupled receptor protein cDNA included in pAH2-17.

EXAMPLES

5 Described below are working examples of the present
invention which are provided only for illustrative purposes,
and not to limit the scope of the present invention. In light
of the present disclosure, numerous embodiments within the
scope of the claims will be apparent to those of ordinary skill
10 in the art.

Example 1

Preparation of Synthetic DNA Primer for Amplifying DNA Coding for G Protein Coupled Receptor Protein

15 A comparison of deoxyribonucleotide sequences
coding for the known amino acid sequences corresponding to
or near the first membrane-spanning domain each of
human-derived TRH receptor protein (HTRHR), human-derived
RANTES receptor protein (L10918, HUMRANTES), human Burkitt's
lymphoma-derived unknown ligand receptor protein (X68149,
20 HSBLR1A), human-derived somatostatin receptor protein
(L14856, HUMSOMAT), rat-derived μ -opioid receptor protein
(U02083, RNU02083), rat-derived κ -opioid receptor protein
(U00442, U00442), human-derived neuromedin B receptor protein
(M73482, HUMNMBR), human-derived muscarinic acetylcholine
25 receptor protein (X15266, HSHM4), rat-derived adrenaline
 α_1 B receptor protein (L08609, RATAADRE01), human-derived
somatostatin 3 receptor protein (M96738, HUMSSTR3X),
human-derived C_5a receptor protein (HUMC5AAR), human-derived
unknown ligand receptor protein (HUMRDC1A), human-derived
30 unknown ligand receptor protein (M84605, HUMOPIODRE) and
rat-derived adrenaline α_2 B receptor protein (M91466,
RATA2BAR) was made. As a result, highly homologous
regions or parts were found (Figure 1).

35 Further, a comparison of deoxynucleotide sequences
coding for the known amino acid sequences corresponding to or
near the sixth membrane-spanning domain each of mouse-derived

unknown ligand receptor protein (M80481, MUSGIR), human-derived bombesin receptor protein (L08893, HUMBOMB3S), human-derived adenosine A2 receptor protein (S46950, S46950), mouse-derived unknown ligand receptor protein (D21061, MUSGPCR), mouse-derived TRH receptor protein (S43387, S43387), rat-derived neuromedin K receptor protein (J05189, RATNEURA), rat-derived adenosine A1 receptor protein (M69045, RATA1ARA), human-derived neurokinin A receptor protein (M57414, HUMNEKAR), rat-derived adenosine A3 receptor protein (M94152, RATADENREC), human-derived somatostatin 1 receptor protein (M81829, HUMSRI1A), human-derived neurokinin 3 receptor protein (S86390, S86371S4), rat-derived unknown ligand receptor protein (X61496, RNCGPCR), human-derived somatostatin 4 receptor protein (L07061, HUMSSTR4Z) and rat-derived GnRH receptor protein (M31670, RATGNRHA) was made. As a result, highly homologous regions or parts were found (Figure 2).

The aforementioned abbreviations in the parentheses are identifiers (reference numbers) which are indicated when GenBank/EMBL Data Bank is retrieved by using DNASIS Gene/Protein Sequencing Data Base (CD019, Hitachi Software Engineering, Japan) and are usually called "Accession Numbers" or "Entry Names". HTRHR is, however, the sequence as disclosed in Japanese Unexamined Patent Publication No. 286986/1993 (EPA 638645).

Specifically, it was planned to incorporate mixed bases relying upon the base regions that were in agreement with cDNAs coding for a large number of receptor proteins in order to enhance base agreement of sequences with as many receptor cDNAs as possible even in other regions. Based upon these sequences, the degenerate synthetic DNA having a nucleotide sequence represented by SEQ ID NO: 1 which is complementary to the homologous nucleotide sequence of Figure 1 and the degenerate synthetic DNA having a nucleotide sequence represented by SEQ ID NO: 2 which is complementary to the homologous nucleotide sequence of Figure 2 were produced. Nucleotide synthesis was carried out by a DNA synthesizer.

[Synthetic DNAs]

5'-CGTGG (G or C) C (A or C) T (G or C) (G or C) TGGGCAAC
(A, G, C or T) (C or T) CCTG-3'

(SEQ ID NO: 1)

5 5'-GT (A, G, C or T) G (A or T) (A or G) (A or G) GGCA
(A, G, C or T) CCAGCAGA (G or T) GGCAAA-3'

(SEQ ID NO: 2)

10 The parentheses indicate the incorporation of a plurality of bases, leading to multiple oligonucleotides in the primer preparation. In other words, nucleotide residues in parentheses of the aforementioned DNAs were incorporated in the presence of a mixture of plural bases at the time of synthesis.

Example 2

15 Isolation of Human Somatostatin Receptor Protein-Encoding DNA,
Human D5 Dopamine Receptor Protein-Encoding DNA, and Rat
Somatostatin Receptor Protein-Encoding DNA

(1) Amplification of DNA by Polymerase Chain Reaction (PCR)
cDNAs (QuickClone, CLONTECH Laboratories, Inc.)

20 prepared from human brain amygdaloid nucleus, human pituitary gland and rat brain each in an amount of 1 ng as templates, the synthetic DNA primers prepared in Example 1 each in an amount of 1 μ M, 2.5 mM dNTPs (deoxyribonucleoside triphosphates), and 2.5 units of Taq DNA polymerase (Takara Shuzo Co., Japan) were mixed together with a buffer attached to the enzyme kit
25 such that the total amount was 100 μ l. The polymerase chain reaction was carried out by using a Thermal Cycler manufactured by Perkin-Elmer Co. One cycle was set to include 96 °C for 30 sec., 45 °C for 1 min. and 60 °C for 3 min.. Totally this one cycle was repeated 30 times to amplify DNAs.

30 Amplification of DNAs was confirmed by 1.2% agarose electrophoresis [Figure 17].

(2) Isolation of Amplified DNA and Analysis of DNA Sequence

By using a TA Cloning Kit (Invitrogen Co.), the DNA amplified by the PCR was inserted into a plasmid vector, pCRTM II.

The DNA was transfected into E. coli attached to the kit to form an amplified DNA library. Colonies formed by the transformants were selected under guidance based on the activity of β -galactosidase on X-gal (5-bromo-4-chloro-3-indolyl- β -D-galactoside)-added LB (Luria-Bertani) plates in order to separate only white colonies in which DNA fragments are inserted. They were cultured in an LB culture medium to which ampicillin was added and plasmid DNAs were prepared with an automatic plasmid extracting machine (Kurabo Co., Japan).

An aliquot of the DNA thus prepared was further digested with EcoRI to confirm DNA fragments that were inserted, and a DNA yield each of clones was compared with a marker. An aliquot of the plasmid DNA thus prepared was treated with RNase, extracted with phenol/chloroform, precipitated in ethanol, and the resulting product was then reacted for sequencing by using a DyeDeoxy terminator cycle sequencing kit (Applied Biosystems Co.).

Sequencing was carried out by using a 370A fluorescent automatic sequencer manufactured by Applied Biosystems Co. The nucleotide sequences obtained were analyzed by using DNASIS (Hitachi Software Engineering, Japan). The nucleotide sequences obtained are shown in Figures 18, 19, 20 and 21. From these Figures and the results of homology retrieval, it was learned that the DNAs obtained were DNAs encoding human somatostatin receptor protein [Figures 18 and 19], human D5 dopamine receptor protein [Figure 20] and rat somatostatin receptor protein [Figure 21] that can be classified each into a group of G protein coupled receptor proteins.

In Figure 18 as described herein, the nucleotide sequence of the DNA is in agreement with the nucleotide sequence encoding somatostatin receptor (HUMSOMAT) and the clone, A58, is a human somatostatin receptor cDNA. The underlined part represents the 5' side synthetic DNA primer

used for the PCR. Thus, even when parts of the nucleotide sequence are mismatched, amplification is effected to a sufficient degree by the PCR.

It will be understood from Figure 19 that the clone, A58 is in good agreement with the nucleotide sequence coding for the human somatostatin receptor (HUMSOMAT) even when the sequencing is carried out from the opposite side. The underlined part represents the 3' side synthetic DNA primer used for the PCR. In this figure, the nucleotide sequences are mismatched to some extent even in the portions other than the primer portion presumably due to base substitution at the time of PCR or due to partial deviation in the sequencing reaction. It can be confirmed via sequencing of chains complementary thereto as required.

In Figure 20 as described herein, the nucleotide sequence of the DNA is in good agreement with a nucleotide sequence coding for the human D5 dopamine receptor (HUMDRD5A) except the primer portion (underlined). It was learned that the clone, 57-A-2, is a human D5 dopamine receptor cDNA.

In Figure 21 as described herein, the DNA is in good agreement with a nucleotide sequence coding for the rat somatostatin receptor (RNU04738) except the primer portion (underlined). It was learned that the clone, B54, is a rat somatostatin receptor cDNA.

Example 3

Isolation of Human Pituitary Gland-Derived G Protein Coupled Receptor Protein-Encoding DNA

(1) Amplification of Receptor cDNA by PCR Using Human Pituitary Gland-Derived cDNA

By using human pituitary gland-derived cDNA (QuickClone, CLONTECH Laboratories, Inc.) as a template, PCR amplification using the DNA primers synthesized in Example 1 was carried out. The composition of the reaction solution consisted of the synthetic DNA primers (SEQ: 5' primer sequence and 3' primer sequence) each in an amount of $1\mu\text{M}$, 1 ng of the template cDNA, 0.25 mM dNTPs, 1 μl of Taq DNA polymerase and

a buffer attached to the enzyme kit, and the total amount of the reaction solution was made to be 100 μ l. The cycle for amplification including 95 °C for 1 min., 55 °C for 1 min. and 72 °C for 1 min. was repeated 30 times by using a Thermal Cycler (Perkin-Elmer Co.). Prior to adding Taq DNA polymerase, the remaining reaction solution was mixed and was heated at 95 °C for 5 minutes and at 65 °C for 5 minutes. The amplified products were confirmed relying upon 1.2% agarose gel electrophoresis and ethidium bromide staining.

(2) Subcloning of PCR Product into Plasmid Vector and Selection of Novel Receptor Candidate Clone via Decoding Nucleotide Sequence of Inserted cDNA Region

The PCR products were separated by using a 0.8% low-melting temperature agarose gel, the band parts were excised from the gel with a razor blade, and were heat-melted, extracted with phenol and precipitated in ethanol to recover DNAs. According to the protocol attached to a TA Cloning Kit (Invitrogen Co.), the recovered DNAs were subcloned into the plasmid vector, pCRTM II (TM represents registered trademark). The recombinant vectors were introduced into E. coli INV α F' competent cells (Invitrogen Co.) to produce transformants. Then, transformant clones having a cDNA-inserted fragment were selected in an LB agar culture medium containing ampicillin and X-gal. Only transformant clones exhibiting white color were picked with a sterilized toothstick to obtain transformant Escherichia coli INV α F'/p19P2.

The individual clones were cultured overnight in an LB culture medium containing ampicillin and treated with an automatic plasmid extracting machine (Kurabo Co., Japan) to prepare plasmid DNAs. An aliquot of the DNA thus prepared was cut by EcoRI to confirm the size of the cDNA fragment that was inserted. An aliquot of the remaining DNA was further processed with RNase, extracted with phenol/chloroform, and precipitated in ethanol so as to be condensed. Sequencing was carried out by using a DyeDeoxy terminator cycle sequencing kit (ABI Co.), the DNAs were decoded by using a

fluorescent automatic sequencer, and the data of the nucleotide sequences obtained were read by using DNASIS (Hitachi System Engineering Co., Japan). The underlined portions represent regions corresponding to the synthetic primers [Figures 22 and 23].

Homology retrieval was carried out based upon the determined nucleotide sequences [Figures 22 and 23]. As a result, it was learned that a novel G protein coupled receptor protein was encoded by the cDNA fragment insert in the plasmid, p19P2, possessed by the transformant Escherichia coli INV α F'/p19P2. To further confirm this fact, by using DNASIS (Hitachi System Engineering Co., Japan) the nucleotide sequences were converted into amino acid sequences [Figures 22 and 23], and homology retrieval was carried out in view of hydrophobicity plotting [Figures 24 and 25] and at the amino acid sequence level to find homology relative to neuropeptide Y receptor proteins [Figure 26].

Example 4

Isolation of Mouse Pancreas-Derived G Protein Coupled Receptor Protein-Encoding DNA

(1) Preparation of Poly(A)⁺ RNA Fraction from Mouse Pancreatic β -Cell Strain, MIN6 and Synthesis of cDNA

A total RNA was prepared from the mouse pancreatic β -cell strain, MIN6 (Jun-ichi Miyazaki et al., Endocrinology, Vol. 127, No. 1, p.126-132) according to the guanidine thiocyanate method (Kaplan B.B. et al., Biochem. J., 183, 181-184 (1979)) and, then, poly(A)⁺ RNA fractions were prepared with a mRNA purifying kit (Pharmacia Co.). Next, to 5 μ g of the poly(A)⁺ RNA fraction was added a random DNA hexamer (BRL Co.) as a primer, and the resulting mixture was subjected to reaction with mouse Moloney Leukemia virus (MMLV) reverse transcriptase (BRL Co.) in the buffer attached to the MMLV reverse transcriptase kit to synthesize complementary DNAs. The reaction product was extracted with phenol/chloroform (1:1), precipitated in ethanol, and was then dissolved in 30 μ l of TE buffer (10 mM Tris-HCl at pH8.0, 1 mM EDTA at pH8.0).

(2) Amplification of Receptor cDNA by PCR Using MIN6-Derived cDNA and Sequencing

By using, as a template, 5 μ l of cDNA prepared from the mouse pancreatic β -cell strain, MIN6 in the above step (1), PCR amplification using the DNA primers synthesized in Example 1 was carried out under the same conditions as in Example 3(2). The resulting PCR product was subcloned into the plasmid vector, pCRTM II, in the same manner as in Example 2 to obtain a plasmid, pG3-2. The plasmid pG3-2 was transfected into E. coli INV α F' to obtain transformed Escherichia coli INV α F'/pG3-2.

By using, as a template, 5 μ l of the cDNA prepared from the mouse pancreatic β -cell strain, MIN6, PCR amplification using DNA primers as disclosed in Libert F. et al., "Science, 244:569-572, 1989", i.e., a degenerate synthetic primer represented by the following sequence:

5'-CTGTG (C or T) G (C or T) (G or C) AT (C or T) GCIIT
(G or T) GA (C or T) (A or C) G (G or C) TAC-3'

(SEQ ID NO: 60)

wherein I is inosine; and
a degenerate synthetic primer represented by the following sequence:

5'-A (G or T) G (A or T) AG (A or T) AGGGCAGCCAGCAGAI
(G or C) (A or G) (C or T) GAA-3'

(SEQ ID NO: 61)

wherein I is inosine,
was carried out under the same conditions as in Working Example 1. The resulting PCR product was subcloned into the plasmid vector, pCRTM II, in the same manner as described in Example 3(2) to obtain a plasmid, pG1-10.

The reaction for determining the nucleotide sequence (sequencing) was carried out with a DyeDeoxy terminator cycle sequencing kit (ABI Co.), the DNA was decoded with the fluorescent automatic sequencer (ABI Co.), and the data

of the nucleotide sequence obtained were analyzed with DNASIS (Hitachi System Engineering Co., Japan).

Figure 27 shows a mouse pancreatic β -cell strain MIN6-derived G protein coupled receptor protein-encoding DNA and an amino acid sequence encoded by the isolated DNA based upon the nucleotide sequences of plasmids pG3-2 and pG1-10 which are held by the transformant Escherichia coli INV α F'/pG3-2. The underlined portions represent regions corresponding to the synthetic primers.

Homology retrieval was carried out based upon the determined nucleotide sequence [Figure 27]. As a result, it was learned that a novel G protein coupled receptor protein was encoded by the cDNA fragment obtained. To further confirm this fact, by using DNASIS (Hitachi System Engineering Co., Japan) the nucleotide sequence was converted into an amino acid sequence [Figure 27], hydrophobicity plotting was carried out to confirm the presence of six hydrophobic regions [Figure 28]. Upon comparing the amino acid sequence with that of p19P2 obtained in Example 3, furthermore, a high degree of homology was found as shown in [Figure 61]. As a result, it is strongly suggested that the G protein coupled receptor proteins encoded by pG3-2 and pG1-10 recognize the same ligand as the G protein coupled receptor protein encoded by p19P2 does while the animal species from which the receptor proteins encoded by pG3-2 and pG1-10 are derived is different from that from which the receptor protein encoded by p19P2 is.

Example 5

Isolation of Human Amygdaloid Nucleus-Derived G Protein Coupled Receptor Protein-Encoding DNA

(1) Amplification of Receptor cDNA by PCR Using Human Amygdaloid Nucleus-Derived cDNA

By using an amplified human amygdala-derived cDNA (QuickClone, CLONTECH Laboratories, Inc.) as a template, PCR amplification using the DNA primers synthesized in Example 1 was carried out. The composition of the reaction solution

consisted of the synthetic DNA primers (SEQ: 5' primer sequence and 3' primer sequence) each in an amount of 1 μ M, 1 ng of the template cDNA, 0.25 mM dNTPs, 1 μ l of Taq DNA polymerase and a buffer attached to the enzyme kit, and the total amount of the reaction solution was made to be 100 μ l. The cycle for amplification including 95 °C for 1 min., 55 °C for 1 min. and 72 °C for 1 min. was repeated 30 times by using a Thermal Cycler (Perkin-Elmer Co.). Prior to adding Taq DNA polymerase, the remaining reaction solution was mixed and was heated at 95 °C for 5 minutes and at 65 °C for 5 minutes. The amplified products were confirmed relying upon 1.2% agarose gel electrophoresis and ethidium bromide staining.

(2) Subcloning of PCR Product into Plasmid Vector and Selection of Novel Receptor Candidate Clone via Decoding Nucleotide Sequence of Inserted cDNA Region

The PCR products were separated by using a 0.8% low-melting temperature agarose gel, the band parts were excised from the gel with a razor blade, and were heat-melted, extracted with phenol and precipitated in ethanol to recover DNAs. According to the protocol attached to a TA Cloning Kit (Invitrogen Co.), the recovered DNAs were subcloned to the plasmid vector, pCRTM II. The recombinant vectors were introduced into E. coli INV α F' competent cells (Invitrogen Co.) to produce transformants. Then, transformant clones having a cDNA-inserted fragment were selected in an LB agar culture medium containing ampicillin and X-gal. Only transformant clones exhibiting white color were picked with a sterilized toothstick to obtain transformant Escherichia coli INV α F'/p63A2.

The individual clones were cultured overnight in an LB culture medium containing ampicillin and treated with an automatic plasmid extracting machine (Kurabo Co., Japan) to prepare plasmid DNAs. An aliquot of the DNA thus prepared was cut by EcoRI to confirm the size of the cDNA fragment that was inserted. An aliquot of the remaining DNA was further processed with RNase, extracted with phenol/chloroform, and

precipitated in ethanol so as to be condensed. Sequencing was carried out by using a DyeDeoxy terminator cycle sequencing kit (ABI Co.), the DNAs were decoded by using a fluorescent automatic sequencer, and the data of the nucleotide sequences obtained were read by using DNASIS (Hitachi System Engineering Co., Japan).

Homology retrieval was carried out based upon the determined nucleotide sequences [Figures 29 and 30]. As a result, it was learned that a novel G protein coupled receptor protein was encoded by the cDNA fragment insert in the plasmid, p63A2 possessed by the transformant Escherichia coli INV α F'/p63A2. To further confirm this fact, by using DNASIS (Hitachi System Engineering Co., Japan) the nucleotide sequences were converted into amino acid sequences [Figures 29 and 30], and homology retrieval was carried out in view of hydrophobicity plotting [Figures 31 and 32] and at the amino acid sequence level to find homology relative to mouse GIR [Figure 33].

Example 6

Cloning of Human Pituitary Gland-Derived G Protein Coupled Receptor Protein cDNA

(1) Cloning of cDNA Comprising Whole Coding Regions for Receptor Protein from Human Pituitary Gland-Derived cDNA Library

The DNA library constructed by Clontech Co. wherein λ gt11 phage vector is used (CLONTECH Laboratories, Inc.; CLH L1139b) was employed as a human pituitary gland-derived cDNA library. The human pituitary gland cDNA library (2×10^6 pfu (plaque forming units)) was mixed with E. coli Y1090⁻ treated with magnesium sulfate, and incubated at 37°C for 15 minutes followed by addition of 0.5% agarose (Pharmacia Co.) LB. The E. coli was plated onto a 1.5% agar (Wako-Junyaku Co.) LB plate (containing 50 μ g/ml of ampicillin). A nitrocellulose filter was placed on the plate on which plaques were formed and the plaque was transferred onto the filter. The filter was denatured with an alkali and

then heated at 80 °C for 3 hours to fix DNAs.

5 The filter was incubated overnight at 42 °C together with the probe mentioned herein below in a buffer containing 50% formamide, 5 x SSPE (20 x SSPE (pH 7.4) is 3 M NaCl, 0.2 M $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, 25 mM EDTA), 5 X Denhardt's solution (Nippon Gene, Japan), 0.1% SDS and 100 μ g/ml of salmon sperm DNA for hybridization.

10 The probe used was obtained by cutting the DNA fragment inserted in the plasmid, p19P2, obtained in Working Example 3, with EcoRI, followed by recovery and labelling by incorporation of [32 P]dCTP (Dupont Co.) with a random prime DNA labelling kit (Amasham Co.).

15 It was washed with 2 x SSC (20 x SSC is 3 M NaCl, 0.3 M sodium citrate), 0.1% SDS at 55 °C for 1 hour and, then, subjected to an autoradiography at -80 °C to detect hybridized plaques.

20 In this screening, hybridization signals were recognized in three independent plaques. Each DNA was prepared from the three clones. The DNAs digested with EcoRI were subjected to an agarose electrophoresis and were analyzed by the southern blotting using the same probe as the one used in the screening. Hybridizing bands were identified at about 0.7kb, 0.8 kb and 2.0kb, respectively. Among them, the DNA fragment corresponding to the band at about 2.0kb (λ hGR3) was selected. The λ hGR3-derived EcoRI fragment with a hybridizable size was subcloned to the EcoRI site of the plasmid, pUC18, and E. coli JM109 was transformed with the plasmid to obtain transformant E. coli JM109/phGR3. A restriction enzyme map of the plasmid, phGR3, was prepared relying upon a restriction enzyme map deduced from the nucleotide sequence as shown in Example 3.

30 As a result, it was learned that it carried a full-length receptor protein-encoding DNA which was predicted from the receptor protein-encoding DNA as shown in Example 3.

(2) Sequencing of Human Pituitary Gland-Derived Receptor Protein cDNA.

Among the EcoRI fragments inserted in the plasmid, phGR3, obtained in the above step (1), the from EcoRI to NheI nucleotide sequence with about 1330bp that is considered to be a receptor protein-coding region was sequenced. Concretely speaking, by utilizing restriction enzyme sites that exist in the EcoRI fragments, unnecessary parts were removed or necessary fragments were subcloned in order to prepare template plasmids for analyzing the nucleotide sequence.

The reaction for determining the nucleotide sequence (sequencing) was carried out with a DyeDeoxy terminator cycle sequencing kit (ABI Co.), the DNA was decoded with the fluorescent automatic sequencer (ABI Co.), and the data of the nucleotide sequence obtained were analyzed with DNASIS (Hitachi System Engineering Co., Japan).

Figure 34 shows a nucleotide sequence of from immediate after the EcoRI site up to the NheI site encoded by phGR3. The nucleotide sequence of the human pituitary gland-derived receptor protein-encoding DNA corresponds to the nucleotide sequence of from 118th to 123rd nucleotides [Figure 34]. An amino acid sequence of the receptor protein that is encoded by the nucleotide sequence is shown in Figure 34. Figure 36 shows the results of hydrophobicity plotting based upon the amino acid sequence.

(3) Northern Hybridization with Human Pituitary Gland-Derived Receptor Protein-Encoding phGR3

Northern blotting was carried out in order to detect the expression of phGR3-encoded human pituitary gland-derived receptor proteins in the pituitary gland at a mRNA level. Human pituitary gland mRNA (2.5 μ g, Clontech Co.) was used as a template mRNA and the same as the probe used in Working Example 5 was used as a probe. Nylon membrane (Pall Biodyne, U.S.A.) was used as a filter for northern blotting and migration of the mRNA and adsorption (sucking) thereof with the blotting filter was carried out according to the method as

disclosed in Molecular Cloning, Cold Spring Harbor Laboratory Press, 1989.

The hybridization was effected by incubating the above-mentioned filter and probe in a buffer containing 50% formamide, 5 x SSPE, 5 X Denhardt's solution, 0.1% SDS and 100 μ g/ml of salmon sperm DNA overnight at 42 °C. The filter was washed with 0.1 x SSC, 0.1% SDS at 50 °C and, after drying with an air, was exposed to an X-ray film (XAR5, Kodak) for three days at -80 °C. The results were as shown in Figure 35 from which it is considered that the receptor gene encoded by phGR3 is expressed in the human pituitary gland.

Example 7

Cloning of Mouse Pancreatic β -Cell Strain, MIN6-Derived G Protein Coupled Receptor Protein cDNA

(1) Preparation of Poly(A)⁺ RNA Fraction from Mouse Pancreatic β -Cell Strain, MIN6 and Synthesis of cDNA

A total RNA was prepared from the mouse pancreatic β -cell strain, MIN6 (Jun-ichi Miyazaki et al., Endocrinology, Vol. 127, No. 1, p.126-132) according to the guanidine thiocyanate method (Kaplan B.B. et al., Biochem. J., 183, 181-184 (1979)) and, then, poly(A)⁺ RNA fractions were prepared with a mRNA purifying kit (Pharmacia Co.). Next, to 5 μ g of the poly(A)⁺ RNA fraction was added a random DNA hexamer (BRL Co.) as a primer, and the resulting mixture was subjected to reaction with MMLV reverse transcriptase (BRL Co.) in the buffer attached to the MMLV reverse transcriptase kit to synthesize complementary DNAs. The reaction product was extracted with phenol/chloroform (1:1), precipitated in ethanol, and was then dissolved in 30 μ l of TE.

(2) Amplification of Receptor cDNA by PCR Using MIN6-Derived cDNA and Sequencing

By using, as a template, 5 μ l of cDNA prepared from the mouse pancreatic β -cell strain, MIN6 in the above step (1), PCR amplification using the DNA primers synthesized in Example 1 was carried out. A reaction solution was

composed of the synthetic DNA primers (SEQ: 5' primer sequence and 3' primer sequence) each in an amount of 100 pM, 0.25 mM dNTPs, 1 μ l of Taq DNA polymerase and 10 μ l of 10 \times buffer attached to the enzyme kit, and the total amount of the reaction solution was made to be 100 μ l. The cycle for amplification including 96 °C for 30 sec., 45 °C for 1 min. and 60 °C for 3 min. was repeated 30 times by using a Thermal Cycler (Perkin-Elmer Co.). Prior to adding Taq DNA polymerase, the remaining reaction solution was mixed and was heated at 95 °C for 5 minutes and at 65 °C for 5 minutes. The amplified products were confirmed relying upon 1.2% agarose gel electrophoresis and ethidium bromide staining.

(3) Subcloning of PCR Product into Plasmid Vector and Selection of Novel Receptor Candidate Clone via Decoding Nucleotide Sequence of Inserted cDNA Region

The PCR products obtained in the above step (2) were separated by using a 0.8% low-melting temperature agarose gel, the band parts were excised from the gel with a razor blade, and were heat-melted, extracted with phenol and precipitated in ethanol to recover DNAs. According to the protocol attached to a TA Cloning Kit (Invitrogen Co.), the recovered DNAs were subcloned to the plasmid vector, pCRTM II. The recombinant vectors were introduced into E. coli JM109 competent cells (Takara Shuzo Co., Japan) to produce transformants. Then, transformant clones having a cDNA-inserted fragment were selected in an LB agar culture medium containing ampicillin, IPTG (isopropylthio- β -D-galactoside) and X-gal. Only transformant clones exhibiting white color were picked with a sterilized toothstick to obtain transformant Escherichia coli JM109/p3H2-17.

The individual clones were cultured overnight in an LB culture medium containing ampicillin and treated with an automatic plasmid extracting machine (Kurabo Co., Japan) to prepare plasmid DNAs. An aliquot of the DNAs thus prepared was cut by EcoRI to confirm the size of the cDNA fragment that was inserted. An aliquot of the remaining DNAs was

further processed with RNase, extracted with phenol/chloroform, and precipitated in ethanol so as to be condensed. Sequencing was carried out by using a DyeDeoxy terminator cycle sequencing kit (ABI Co.), the DNAs were decoded by using a fluorescent automatic sequencer, and the data of the nucleotide sequences obtained were read by using DNASIS (Hitachi System Engineering Co., Japan).

Homology retrieval was carried out based upon the determined nucleotide sequence [Figure 37]. As a result, it was learned that a novel G protein coupled receptor protein was encoded by the cDNA fragment insert in the plasmid possessed by the transformant Escherichia coli JM109/p3H2-17. To further confirm this fact, by using DNASIS (Hitachi System Engineering Co., Japan) the nucleotide sequence were converted into an amino acid sequence [Figure 37], and homology retrieval was carried out in view of hydrophobicity plotting [Figure 38] and at the amino acid sequence level to find homology relative to chicken ATP receptor (P34996), human somatostatin receptor subtype 3 (A46226), human somatostatin receptor subtype 4 (JN0605) and bovine neuropeptide Y receptor (S28787) [Figure 39]. Abbreviations in parentheses are reference numbers assigned when they are registered as data to NBRF-PIR/Swiss-PROT and are usually called "Accession Numbers".

Example 8

Cloning of Mouse Pancreatic β -Cell Strain, MIN6-Derived G Protein Coupled Receptor Protein cDNA

(1) Preparation of Poly(A)⁺ RNA Fraction from Mouse Pancreatic β -Cell Strain, MIN6 and Synthesis of cDNA

A total RNA was prepared from the mouse pancreatic β -cell strain, MIN6 (Jun-ichi Miyazaki et al., Endocrinology, Vol. 127, No. 1, p.126-132) according to the guanidine thiocyanate method (Kaplan B.B. et al., Biochem. J., 183, 181-184 (1979)) and, then, poly(A)⁺ RNA fractions were prepared with a mRNA purifying kit (Pharmacia Co.). Next, to 5 μ g of the poly(A)⁺ RNA fraction was added a random DNA hexamer (BRL Co.) as a primer, and the resulting mixture was subjected

to reaction with MMLV reverse transcriptase (BRL Co.)
in the buffer attached to the MMLV reverse transcriptase kit
to synthesize complementary DNAs. The reaction product
was extracted with phenol/chloroform (1:1), precipitated in
5 ethanol, and was then dissolved in 30 μ l of TE.

(2) Amplification of Receptor cDNA by PCR Using MIN6-Derived
cDNA and Sequencing

By using, as a template, 5 μ l of cDNA prepared
from the mouse pancreatic β -cell strain, MIN6, in the above
10 step (1), PCR amplification using the DNA primers synthesized
in Example 1 was carried out. A reaction solution was
composed of the synthetic DNA primers (SEQ: 5' primer sequence
and 3' primer sequence) each in an amount of 100 pM,
0.25 mM dNTPs, 1 μ l of Taq DNA polymerase and 10 μ l of 10 \times
15 buffer attached to the enzyme kit, and the total amount of the
reaction solution was made to be 100 μ l. The cycle for
amplification including 96 °C for 30 sec., 45 °C for 1 min.
and 60 °C for 3 min. was repeated 30 times by using a Thermal
Cycler (Perkin-Elmer Co.). Prior to adding Taq DNA
20 polymerase, the remaining reaction solution was mixed and was
heated at 95 °C for 5 minutes and at 65 °C for 5 minutes.
The amplified products were confirmed relying upon 1.2%
agarose gel electrophoresis and ethidium bromide staining.

(3) Subcloning of PCR Product into Plasmid Vector and
25 Selection of Novel Receptor Candidate Clone via Decoding
Nucleotide Sequence of Inserted cDNA Region

The PCR products obtained in the above step (2)
were separated with a 0.8% low-melting temperature agarose
gel, the band parts were excised from the gel with a razor
30 blade, and were heat-melted, extracted with phenol and
precipitated in ethanol to recover DNAs. According to the
protocol attached to a TA Cloning Kit (Invitrogen Co.),
the recovered DNAs were subcloned to the plasmid vector,
pCRTM II. The recombinant vectors were introduced into
35 E. coli JM109 competent cells (Takara Shuzo Co., Japan)

to produce transformants. Then, transformant clones having a cDNA-inserted fragment were selected in an LB agar culture medium containing ampicillin, IPTG and X-gal. Only transformant clones exhibiting white color were picked with a sterilized toothstick to obtain transformant Escherichia coli JM109/p3H2-34.

The individual clones were cultured overnight in an LB culture medium containing ampicillin and treated with an automatic plasmid extracting machine (Kurabo Co., Japan) to prepare plasmid DNAs. An aliquot of the DNAs thus prepared was cut by EcoRI to confirm the size of the cDNA fragment that was inserted. An aliquot of the remaining DNAs was further processed with RNase, extracted with phenol/chloroform, and precipitated in ethanol so as to be condensed. Sequencing was carried out by using a DyeDeoxy terminator cycle sequencing kit (ABI Co.), the DNAs were decoded by using a fluorescent automatic sequencer, and the data of the nucleotide sequences obtained were read by using DNASIS (Hitachi System Engineering Co., Japan).

Homology retrieval was carried out based upon the determined nucleotide sequence [Figure 40]. As a result, it was learned that a novel G_i protein coupled receptor protein was encoded by the cDNA fragment insert in the plasmid possessed by the transformant Escherichia coli JM109/p3H2-34. To further confirm this fact, by using DNASIS (Hitachi System Engineering Co., Japan) the nucleotide sequence were converted into an amino acid sequence [Figure 40], and homology retrieval was carried out in view of hydrophobicity plotting [Figure 41] and at the amino acid sequence level to find homology relative to human somatostatin receptor subtype 2 (B41795) and rat-derived ligand unknown receptor (A39297) [Figure 42]. Abbreviations in parentheses are reference numbers assigned when they are registered as data to NBRF-PIR/Swiss-PROT and are usually called "Accession Numbers" or "Entry Names".

Example 9

Cloning of Rabbit Gastropyrolic Part Smooth Muscle-Derived G
Protein Coupled Receptor Protein cDNA

(1) Preparation of Poly(A)⁺ RNA Fraction from Rabbit

5 Gastropyrolic Part Smooth Muscle and Synthesis of cDNA

A total RNA was prepared from rabbit gastropyrolic
part smooth muscles by the guanidine thiocyanate method
(Kaplan B.B. et al., Biochem. J. 183, 181-184 (1979)) and,
then, poly(A)⁺ RNA fractions were prepared with a mRNA
10 purifying kit (Pharmacia Co.). Next, to 5 μ g of the
poly(A)⁺ RNA fraction was added a random DNA hexamer
(BRL Co.) as a primer, and the resulting mixture was subjected
to reaction with MMLV reverse transcriptase (BRL Co.)
in the buffer attached to the MMLV reverse transcriptase kit
15 to synthesize complementary DNAs. The reaction product
was extracted with phenol/chloroform (1:1), precipitated in
ethanol, and was then dissolved in 30 μ l of TE (Tris-EDTA
solution).

(2) Amplification of Receptor cDNA by PCR Using Rabbit
20 Gastropyrolic Part Smooth Muscle-Derived cDNA and
Sequencing

By using, as a template, 1 μ l of cDNA prepared
from the rabbit gastropyrolic part smooth muscle in the above
step (1), PCR amplification using the DNA primers synthesized
25 in Example 1 was carried out. A reaction solution
was composed of the synthetic DNA primers (SEQ: 5' primer
sequence and 3' primer sequence) each in an amount of 100 pM,
0.25 mM dNTPs, 1 μ l of Taq DNA polymerase and 10 μ l of
buffer attached to the enzyme kit, and the total amount of the
30 reaction solution was made to be 100 μ l. The cycle for
amplification including 96 °C for 30 sec., 45 °C for 1 min.
and 60 °C for 3 min. was repeated 25 times by using a Thermal
Cycler (Perkin-Elmer Co.). The amplified products were
35 confirmed relying upon 1.2% agarose gel electrophoresis and
ethidium bromide staining.

(3) Subcloning of PCR Product into Plasmid Vector and
Selection of Novel Receptor Candidate Clone via Decoding
Nucleotide Sequence of Inserted cDNA Region

The PCR products obtained in the above step (2)
5 were separated with a 1.0% low-melting temperature agarose
gel, the band parts were excised from the gel with a razor
blade, and were heat-melted, extracted with phenol and
precipitated in ethanol to recover DNAs. According to the
protocol attached to a TA Cloning Kit (Invitrogen Co.),
10 the recovered DNAs were subcloned to the plasmid vector,
pCRTM II. The recombinant vectors were introduced into
E. coli JM109 competent cells (Takara Shuzo Co., Japan) to
produce transformants. Then, transformant clones having
a cDNA-inserted fragment were selected in an LB agar
15 culture medium containing ampicillin, IPTG and X-gal. Only
transformant clones exhibiting white color were picked with
a sterilized toothstick to obtain transformant Escherichia
coli JM109/pMD4.

The individual clones were cultured overnight in an
20 LB culture medium containing ampicillin and treated with an
automatic plasmid extracting machine (Kurabo Co., Japan) to
prepare plasmid DNAs. An aliquot of the DNAs thus prepared
was cut by EcoRI to confirm the size of the cDNA fragment
that was inserted. An aliquot of the remaining DNAs was
25 further processed with RNase, extracted with phenol/chloroform,
and precipitated in ethanol so as to be condensed. Sequencing
was carried out by using a DyeDeoxy terminator cycle
sequencing kit (ABI Co.), the DNAs were decoded by using a
fluorescent automatic sequencer, and the data of the
30 nucleotide sequences obtained were read by using DNASIS
(Hitachi System Engineering Co., Japan). The determined
nucleotide sequence was as shown in Figure 43. It was learned
from Figure 43 that the cloned cDNA fragment was amplified
from both sides with only the synthetic DNA primer having a
35 nucleotide sequence represented by SEQ ID NO: 1 as synthesized
in Example 1.

Homology retrieval was carried out based upon the

determined nucleotide sequence [Figure 43]. As a result, it was learned that a novel G protein coupled receptor protein was encoded by the cDNA fragment insert in the plasmid possessed by the transformant Escherichia coli JM109/pMD4.

5 To further confirm this fact, by using DNASIS (Hitachi System Engineering Co., Japan) the nucleotide sequence were converted into an amino acid sequence [Figure 43], and homology retrieval was carried out in view of hydrophobicity plotting [Figure 44] and at the amino acid sequence level to find homology relative
10 to rat ligand-unknown receptor protein (A35639) [Figure 45]. Abbreviations in parentheses are reference numbers assigned when they are registered as data to NBRF-PIR/Swiss-PROT and are usually called "Accession Numbers".

Example 10

15 Cloning of cDNA Comprising Whole Coding Regions for Receptor Protein from Mouse Pancreatic β -Cell Strain, MIN6-Derived cDNA Library

(1) Cloning of cDNA Comprising Whole Coding Regions for Receptor Protein from Mouse Pancreatic β -Cell Strain,
20 MIN6-Derived cDNA Library

SuperscriptTM Lambda System (BRL, Cat. 8256) distributed by BRL Co. and GigaPack II Gold (Stratagene, Cat. 200215) distributed by Stratagene Co. were used to construct MIN6-derived cDNA libraries. By using the above
25 kits, a MIN6 cDNA library with 2.2×10^6 pfu (plaque forming units) was constructed from 10μ g of MIN6 poly(A)⁺ RNA. The cDNA library was mixed with E. coli Y1090⁻ treated with magnesium sulfate, and incubated at 37 °C for 15 minutes followed by addition of 0.5% agarose (Pharmacia Co.) LB.
30 The E. coli was plated onto a 1.5% agar (Wako-Junyaku Co.) LB plate (containing 50 μ g/ml of ampicillin). A nitrocellulose filter was placed on the plate on which plaques were formed and the plaque was transferred onto the filter. The filter was denatured with an alkali and then heated at 80 °C for 3 hours
35 to fix DNAs.

The filter was incubated overnight at 42 °C together

with the probe mentioned herein below in a buffer containing 50% formamide, 5 x SSPE, 5 X Denhardt's solution, 0.1% SDS and 100 μ g/ml of salmon sperm DNA for hybridization.

5 The probe used was obtained by cutting the DNA fragment inserted in the plasmid, p3H2-34, obtained in Working Example 8, with EcoRI, followed by recovery and labeling by incorporation of [32 P]dCTP (Dupont Co.) with a random prime DNA labelling kit (Amasham Co.).

10 It was washed with 2 x SSC (150 mM NaCl and 15 mM sodium citrate), 0.1% SDS at 55 °C for 1 hour and, then, subjected to an autoradiography at -80 °C to detect hybridized plaques.

15 In this screening, hybridization signals were recognized in two independent plaques. Each DNA was prepared from the two clones. The DNAs digested with SalI and NotI were subjected to an agarose electrophoresis and were analyzed. Inserted fragments were identified at about 2.0kb and 3.0kb, respectively. Between them, the DNA fragment corresponding to the band at about 3.0kb (λ No.20) was selected. The λ No.20-derived NotI-SalI fragment with about 3.0kb was subcloned into the NotI-SalI site of the plasmid, pBluescriptTM II SK(+), and E. coli JM109 was transformed with the plasmid to obtain a transformant E. coli JM109/pMGR20. A restriction enzyme map of the 25 plasmid, pMGR20, was prepared relying upon a restriction enzyme map deduced from the nucleotide sequence as shown in Working Example 8. As a result, it was learned that it carried a full-length receptor protein-encoding DNA which was predicted from the receptor protein-encoding DNA as shown in Working 30 Example 8.

(2) Sequencing of MIN6-Derived Receptor Protein Full-Length CDNA

35 Among the NotI-SalI fragments inserted in the plasmid, pMGR20, obtained in the above step (1), the nucleotide sequence with total 1607bp, including not only a region that is considered to be a receptor protein-coding region (ORF) but

also a neighboring region thereof was sequenced. Concretely speaking, by utilizing restriction enzyme sites that exist in the NotI-SalI fragments, unnecessary parts were removed or necessary fragments were subcloned in order to prepare
5 template plasmids for analyzing the nucleotide sequence thereof. As for the nucleotide sequences of part of the regions, primers for sequencing were synthesized based upon the nucleotide sequences that were determined already and used to make confirmation.

10 The reaction for determining the nucleotide sequence (sequencing) was carried out with a DyeDeoxy terminator cycle sequencing kit (ABI Co.), the DNA was decoded with the fluorescent automatic sequencer (ABI Co.), and the data of the nucleotide sequence obtained were analyzed with DNASIS
15 (Hitachi System Engineering Co., Japan).

Figure 46 shows a nucleotide sequence around an open reading frame (ORF) of a mouse galanin receptor protein encoded by the cDNA insert in pMGR20. The nucleotide sequence of mouse galanin receptor protein-encoding DNA
20 corresponds to from the 481st to 1525th nucleotides of the nucleotide sequence in Figure 46. The nucleotide sequence was converted into an amino acid sequence [Figure 46] and hydrophobicity plotting was carried out [Figure 47]. Since the amino acid sequence [Figure 46] has 92% homology to the
25 human-derived galanin receptor protein at the amino acid sequence level [Figure 48], it was learned that the cDNA insert in the pMGR20 is a mouse-derived galanin receptor protein-encoding cDNA.

Example 11

30 Preparation of Synthetic DNA Primer for Amplifying G Protein Coupled Receptor Protein-Encoding DNA

Highly homologous parts were found by comparing nucleotide sequences corresponding to or near the third membrane-spanning domain [3C and 3D in Figure 4] and the sixth
35 membrane-spanning domain [6C of Figure 6] among known G protein coupled receptors, i.e., rat-derived angiotensin II

receptor protein (L32840), rat-derived angiotensin Ib receptor
protein (X64052), rat-derived angiotensin receptor protein
subtype (M90065), human-derived angiotensin Ia receptor protein
(M91464), rat-derived cholecystokinin_A receptor protein
5 (M88096), rat-derived cholecystokinin_B receptor protein
(M99418), human-derived cholecystokinin_B receptor protein
(L04473), mouse-derived low-affinity interleukin 8 receptor
protein (M73969), human-derived high-affinity interleukin 8
receptor protein (X65858), mouse-derived C5a anaphylatoxin
10 receptor protein (S46665), human-derived N-formyl peptide
receptor protein (M60626), etc.

The aforementioned abbreviations in parentheses are
reference numbers that are indicated when the GenBank/EMBL
data base is retrieved, and are usually called "Accession
15 Numbers".

It was planned to incorporate mixed bases relying
upon the base regions that were in agreement with a large
number of receptor protein cDNAs in order to enhance base
agreement of sequences with as many receptor cDNAs as possible
20 even in other regions. Based upon these sequences, the
degenerate synthetic DNA (3D of Figure 4) having a nucleotide
sequence represented by SEQ ID NO: 3 which is complementary to
the homologous nucleotide sequence of Figure 4 and the
degenerate synthetic DNA (nucleotide sequence complementary to
25 6C of Figure 6) having a nucleotide sequence represented by
SEQ ID NO: 4 were produced. Nucleotide synthesis was carried
out by a DNA synthesizer.

[Synthetic DNAs]

5'-CTCGC (G or C) GC (C or T) (A or C) TI (A or G) G
30 (C or T) ATGGA (C or T) CGITAT-3'

(SEQ ID NO:3)

5'-CATGT (A or G) G (T or A) AGGGAAICCCAG (G or C) A
(A or C) AI (A or G) A (A or G)(A or G) AA-3'

(SEQ ID NO:4)

The parentheses indicate the incorporation of a plurality of bases, leading to multiple oligonucleotides in the primer preparation. In other words, nucleotide residues in parentheses of the aforementioned DNAs were incorporated in the presence of a mixture of plural bases at the time of synthesis, provided that I denotes inosine.

Example 12

Cloning of Rabbit Gastropyrolic Part Smooth Muscle-Derived G Protein Coupled Receptor Protein cDNA

(1) Preparation of Poly(A)⁺ RNA Fraction from Rabbit Gastropyrolic Part Smooth Muscle and Synthesis of cDNA

A total RNA was prepared from rabbit gastropyrolic part smooth muscles by the guanidine thiocyanate method (Kaplan B.B. et al., Biochem. J. 183, 181-184 (1979)) and, then, poly(A)⁺ RNA fractions were prepared with a mRNA purifying kit (Pharmacia Co.). Next, to 5 μ g of the poly(A)⁺ RNA fraction was added a random DNA hexamer (BRL Co.) as a primer, and the resulting mixture was subjected to reaction with MMLV reverse transcriptase (BRL Co.) in the buffer attached to the MMLV reverse transcriptase kit to synthesize complementary DNAs. The reaction product was extracted with phenol/chloroform (1:1), precipitated in ethanol, and was then dissolved in 30 μ l of TE.

(2) Amplification of Receptor cDNA by PCR Using Rabbit Gastropyrolic Part Smooth Muscle-Derived cDNA and Sequencing

By using, as a template, 1 μ l of cDNA prepared from the rabbit gastropyrolic part smooth muscle in the above step (1), PCR amplification using the DNA primer having a nucleotide sequence represented by SEQ ID NO: 3 and the DNA primer having a nucleotide sequence represented by SEQ ID NO: 4 synthesized in Example 11 was carried out. A reaction solution was composed of the synthetic DNA primers (SEQ: 5' primer sequence and 3' primer sequence) each in an amount of 100 pM, 0.25 mM dNTPs, 1 μ l of Taq DNA polymerase

and 10 μ l of buffer attached to the enzyme kit, and the total amount of the reaction solution was made to be 100 μ l. The cycle for amplification including 96 °C for 30 sec., 45 °C for 1 min. and 60 °C for 3 min. was repeated 25 times by using a Thermal Cycler (Perkin-Elmer Co.). The amplified products were confirmed relying upon 1.2% agarose gel electrophoresis and ethidium bromide staining.

(3) Subcloning of PCR Product into Plasmid Vector and Selection of Novel Receptor Candidate Clone via Decoding Nucleotide Sequence of Inserted cDNA Region

The PCR products obtained in the above step (2) were separated with a 1.0% low-melting temperature agarose gel, the band parts were excised from the gel with a razor blade, and were heat-melted, extracted with phenol and precipitated in ethanol to recover DNAs. According to the protocol attached to a TA Cloning Kit (Invitrogen Co.), the recovered DNAs were subcloned to the plasmid vector, pCRTM II. The recombinant vectors were introduced into E. coli JM109 competent cells (Takara Shuzo Co., Japan) to produce transformants. Then, transformant clones having a cDNA-inserted fragment were selected in an LB agar culture medium containing ampicillin, IPTG and X-gal. Only transformant clones exhibiting white color were picked with a sterilized toothstick to obtain transformant Escherichia coli JM109/pMJ10.

The individual clones were cultured overnight in an LB culture medium containing ampicillin and treated with an automatic plasmid extracting machine (Kurabo Co., Japan) to prepare plasmid DNAs. An aliquot of the DNAs thus prepared was cut by EcoRI to confirm the size of the cDNA fragment that was inserted. An aliquot of the remaining DNAs was further processed with RNase, extracted with phenol/chloroform, and precipitated in ethanol so as to be condensed. Sequencing was carried out by using a DyeDeoxy terminator cycle sequencing kit (ABI Co.), the DNAs were decoded by using a fluorescent automatic sequencer, and the data of the

nucleotide sequences obtained were read by using DNASIS (Hitachi System Engineering Co., Japan). The determined nucleotide sequence was as shown in Figure 49.

Homology retrieval was carried out based upon the determined nucleotide sequence [Figure 49]. As a result, it was learned that a novel G protein coupled receptor protein was encoded by the cDNA fragment insert in the plasmid possessed by the transformant Escherichia coli JM109/pMJ10. To further confirm this fact, by using DNASIS (Hitachi System Engineering Co., Japan) the nucleotide sequence were converted into an amino acid sequence [Figure 49], and homology retrieval was carried out in view of hydrophobicity plotting [Figure 50] and at the amino acid sequence level to find homology relative to human ligand unknown receptor protein (B42009), human N-formyl peptide receptor protein (JC2014), rabbit N-formyl peptide receptor protein (A46520), mouse C5a anaphylatoxin receptor protein (A46525) and bovine neuropeptide Y receptor protein (S28787) [Figure 51]. Abbreviations in parentheses are reference numbers assigned when they are registered as data to NBRF-PIR/Swiss-PROT and are usually called "Accession Numbers".

Example 13

Preparation of Synthetic DNA Primer for Amplifying G Protein Coupled Receptor Protein-Encoding DNA

A comparison of nucleotide sequences coding for regions corresponding to or near the third membrane-spanning domain among known G protein coupled receptors, i.e., mouse-derived κ -opioid receptor protein (L11064), mouse-derived δ -opioid receptor protein (L11065), rat-derived μ -opioid receptor protein (D16349), mouse-derived bradykinin B2 receptor protein (X69676), rat-derived bradykinin B2 receptor protein (M599967), mouse-derived bombesin receptor protein (M35328), human-derived neuromedin B receptor protein (M73482), human-derived gastrin releasing peptide receptor protein (M73481), human-derived bombesin receptor protein subtype 3 (L08893), mouse-derived substance K receptor protein

(X62933), mouse-derived substance P receptor protein (X62934), rat-derived neurokinin 3 receptor protein (J05189), rat-derived endothelin receptor protein (M60786), rat-derived ligand unknown receptor proteins (L04672), (X61496), (X59249) and (L09249), mouse-derived ligand unknown receptor protein (P30731), human-derived ligand unknown receptor proteins (M31210) and (U03642), etc. was made. In particular, the degenerate DNA primer having a nucleotide sequence (3B in Figure 3; SEQ ID NO: 6) with highly common bases (highly homologous nucleotides) was synthesized to enhance base agreement of sequences with as many receptor cDNAs as possible even in other regions on the basis of nucleotide sequence regions that were in agreement with a large number of receptor cDNAs. Nucleotide synthesis was carried out by a DNA synthesizer.

The nucleotide sequence represented by SEQ ID NO: 6 is:

5'-CTGAC (C or T) G (C or T) TCTI (A or G)(G or C) I
(A or G)(C or T) TGAC (A or C) G (A, C or G) TAT-3'

The parentheses indicate the incorporation of a plurality of bases, leading to multiple oligonucleotides in the primer preparation. In other words, nucleotide residues in parentheses of the aforementioned DNAs were incorporated in the presence of a mixture of plural bases at the time of synthesis, provided that I denotes inosine.

Furthermore, a comparison of nucleotide sequences coding for regions corresponding to or near the sixth membrane-spanning domain among known G protein coupled receptors, i.e., mouse-derived κ -opioid receptor protein (L11064), mouse-derived δ -opioid receptor protein (L11065), rat-derived μ -opioid receptor protein (D16349), mouse-derived bradykinin B2 receptor protein (X69676), rat-derived bradykinin B2 receptor protein (M59967), mouse-derived bombesin receptor protein (M35328), human-derived neuromedin B receptor protein

(M73482), human-derived gastrin releasing peptide receptor protein (M73481), human-derived bombesin receptor protein subtype 3 (L08893), mouse-derived substance K receptor protein (X62933), mouse-derived substance P receptor protein (X62934),
5 rat-derived neurokinin 3 receptor protein (J05189), rat-derived endothelin receptor protein (M60786), rat-derived ligand unknown receptor proteins (L04672), (X61496), (X59249) and (L09249), mouse-derived ligand unknown receptor protein (P30731), human-derived ligand unknown receptor proteins
10 (M31210) and (U03642), etc. was made. In particular, the degenerate DNA primer having a nucleotide sequence (SEQ ID NO: 8) which is complementary to the nucleotide sequence (6A in Figure 5) with highly common bases (highly homologous nucleotides) was synthesized to enhance base
15 agreement of sequences with as many receptor cDNAs as possible even in other portions on the basis of base regions that are in agreement with a large number of receptor cDNAs.

The nucleotide sequence represented by SEQ ID NO: 8 is:

20 5'-GATGTG (A or G) TA (A or G) GG (G or C)(A or G)
ICCAACAGAIG (A or G) (C or T) AAA-3'

The parentheses indicate the incorporation of a plurality of bases, leading to multiple oligonucleotides in the primer preparation. In other words, nucleotide residues in
25 parentheses of the aforementioned DNAs were incorporated in the presence of a mixture of plural bases at the time of synthesis, provided that I denotes inosine.

The aforementioned abbreviations in parentheses are reference numbers indicated when the GenBank/EMBL data base is
30 retrieved and are usually called "Accession Numbers".

Example 14

Cloning of Rabbit Gastropyrolic Part Smooth Muscle-Derived G
Protein Coupled Receptor Protein cDNA

(1) Preparation of Poly(A)⁺ RNA Fraction from Rabbit

5 Gastropyrolic Part Smooth Muscle and Synthesis of cDNA

A total RNA was prepared from rabbit gastropyrolic part smooth muscles by the guanidine thiocyanate method (Kaplan B.B. et al., Biochem. J. 183, 181-184 (1979)) and, then, poly(A)⁺ RNA fractions were prepared with a mRNA purifying kit (Pharmacia Co.). Next, to 5 μ g of the poly(A)⁺ RNA fraction was added a random DNA hexamer (BRL Co.) as a primer, and the resulting mixture was subjected to reaction with MMLV reverse transcriptase (BRL Co.) in the buffer attached to the MMLV reverse transcriptase kit to synthesize complementary DNAs. The reaction product was extracted with phenol/chloroform (1:1), precipitated in ethanol, and was then dissolved in 30 μ l of TE.

(2) Amplification of Receptor cDNA by PCR Using Rabbit
20 Gastropyrolic Part Smooth Muscle-Derived cDNA and
 Sequencing

By using, as a template, 1 μ l of cDNA prepared from the rabbit gastropyrolic part smooth muscle in the above step (1), PCR amplification using the DNA primer having a nucleotide sequence represented by SEQ ID NO: 6 and the DNA primer having a nucleotide sequence represented by SEQ ID NO: 8 synthesized in Example 13 was carried out.

A reaction solution was composed of the synthetic DNA primers (SEQ: 5' primer sequence and 3' primer sequence) each in an amount of 100 pM, 0.25 mM dNTPs, 1 μ l of Taq DNA polymerase and 10 μ l of buffer attached to the enzyme kit, and the total amount of the reaction solution was made to be 100 μ l. The cycle for amplification including 96 °C for 30 sec., 45 °C for 1 min. and 60 °C for 3 min. was repeated 25 times by using a Thermal Cycler (Perkin-Elmer Co.). The amplified products were confirmed relying upon 1.2% agarose gel electrophoresis and ethidium bromide staining.

(3) Subcloning of PCR Product into Plasmid Vector and
Selection of Novel Receptor Candidate Clone via Decoding
Nucleotide Sequence of Inserted cDNA Region

The PCR products obtained in the above step (2)
5 were separated by using a 1.0% low-melting temperature agarose
gel, the band parts were excised from the gel with a razor
blade, and were heat-melted, extracted with phenol and
precipitated in ethanol to recover DNAs. According to the
protocol attached to a TA Cloning Kit (Invitrogen Co.),
10 the recovered DNAs were subcloned to the plasmid vector,
pCRTM II. The recombinant vectors were introduced into
E. coli JM109 competent cells (Takara Shuzo Co., Japan) to
produce transformants. Then, transformant clones having
a cDNA-inserted fragment were selected in an LB agar
15 culture medium containing ampicillin, IPTG and X-gal. Only
transformant clones exhibiting white color were picked with
a sterilized toothstick to obtain transformant Escherichia
coli JM109/pMH28.

The individual clones were cultured overnight in an
20 LB culture medium containing ampicillin and treated with an
automatic plasmid extracting machine (Kurabo Co., Japan) to
prepare plasmid DNAs. An aliquot of the DNAs thus prepared
was cut by EcoRI to confirm the size of the cDNA fragment
that was inserted. An aliquot of the remaining DNAs was
25 further processed with RNase, extracted with phenol/chloroform,
and precipitated in ethanol so as to be condensed. Sequencing
was carried out by using a DyeDeoxy terminator cycle
sequencing kit (ABI Co.), the DNAs were decoded by using a
fluorescent automatic sequencer, and the data of the
30 nucleotide sequences obtained were read by using DNASIS
(Hitachi System Engineering Co., Japan). The determined
nucleotide sequence was as shown in Figure 52.

Homology retrieval was carried out based upon the
determined nucleotide sequence [Figure 52]. As a result,
35 it was learned that a novel G protein coupled receptor
protein was encoded by the cDNA fragment insert in the
plasmid possessed by the transformant Escherichia coli

JM109/pMH28. To further confirm this fact, by using DNASIS (Hitachi System Engineering Co., Japan) the nucleotide sequence were converted into an amino acid sequence [Figure 52], and homology retrieval was carried out in view of hydrophobicity plotting [Figure 53] and at the amino acid sequence level to find homology relative to mouse IL-8 receptor protein (P35343), human somatostatin receptor protein 1 (A41795) and human somatostatin receptor protein 4 (A47457)[Figure 54]. The aforementioned abbreviations in parentheses are reference numbers assigned when they are registered as data to NBRF-PIR or SWISS-PROT and are usually called "Accession Numbers".

Example 15

Preparation of Synthetic DNA Primer for Amplifying G Protein Coupled Receptor Protein-Encoding DNA

A comparison of nucleotide sequences coding for regions corresponding to or near the second membrane-spanning domain among known G protein coupled receptors, i.e., human-derived galanin receptor (HUMGALAREC), rat-derived α -1B-adrenergic receptor (RATADR1B), human-derived β -1-adrenergic receptor (HUMADRB1), rabbit-derived IL-8 receptor (RABIL8RSB), human-derived opioid receptor (HUMOPIODRE), bovine-derived substance K receptor (BTSKR), human-derived somatostatin receptor-2 (HUMSTRI2A), human-derived somatostatin receptor-3 (HUMSSTR3Y), human-derived gastrin receptor (HUMGARE), human-derived cholecystokinin A receptor (HUMCCKAR), human-derived dopamine receptor-D5 (HUMD1B), human-derived serotonin receptor 5HT1E (HUM5HT1E), human-derived dopamine receptor D4 (HUMD4C), mouse-derived serotonin receptor-2 (MMSERO), rat-derived α -1A-adrenergic receptor (RATADRA1A), rat-derived histamine H2 receptor (S57565), etc. was made. In particular, the degenerate DNA primer having a nucleotide sequence (T2A in Figure 7, SEQ ID NO: 10) with highly common bases (highly homologous nucleotides) was synthesized to enhance base agreement of sequences with as many receptor cDNAs as possible even in other regions on the basis of nucleotide sequence

regions that were in agreement with a large number of receptor cDNAs. Nucleotide synthesis was carried out by a DNA synthesizer.

The nucleotide sequence represented by SEQ ID NO: 10 is:

5'-GYCACCAACN₂WSTTCATCCTSWN₂HCTG-3'

wherein S represents G or C; Y represents C or T; W represents A or T; H represents A, C or T and N₂ represents I.

The parentheses indicate the incorporation of a plurality of bases, leading to multiple oligonucleotides in the primer preparation. In other words, nucleotide residues in parentheses of the aforementioned DNAs were incorporated in the presence of a mixture of plural bases at the time of synthesis, provided that I denotes inosine.

Furthermore, a comparison of nucleotide sequences coding for regions corresponding to or near the seventh membrane-spanning domain among known G protein coupled receptors, i.e., human-derived galanin receptor (HUMGALAREC), rat-derived A1 adenosine receptor (RAT1ADREC), porcine-derived angiotensin receptor (PIGA2R), rat-derived serotonin receptor (RAT5HTRTC), human-derived dopamine receptor (S58541), human-derived gastrin releasing peptide receptor (HUMGRPR), mouse-derived GRP/bombesin receptor (MUSGRPBOM), rat-derived vascular type 1 angiotensin receptor (RRVT1AIIR), human-derived muscarinic acetylcholine receptor (HSHM4), human-derived β -1 adrenergic receptor (HUMDRB1), human-derived gastrin receptor (HUMGARE), rat-derived cholecystokinin receptor (RATCCKAR), rat-derived ligand unknown receptor (S59748), human-derived somatostatin receptor (HUMSST28A), rat-derived ligand unknown receptor (RNGPROCR), mouse-derived somatostatin receptor 1 (MUSSRI1A), human-derived α -A1-adrenergic receptor (HUMA1AADR), mouse-derived delta-opioid receptor (S66181), human-derived somatostatin

receptor-3 (HUMSSTR3Y), etc. was made. In particular, the degenerate DNA primer having a nucleotide sequence (T7A in Figure 8, SEQ ID NO: 11) with highly common bases (highly homologous nucleotides) was synthesized to enhance base agreement of sequences with as many receptor cDNAs as possible even in other regions on the basis of nucleotide sequence regions that were in agreement with a large number of receptor cDNAs. Nucleotide synthesis was carried out by a DNA synthesizer.

The nucleotide sequence represented by SEQ ID NO: 11 is:

5'-ASN₂SAN₂RAAGSARTAGAN₂GAN₂RGGRIT-3'

wherein R represents A or G; S represents G or C and N₂ represents I.

The parentheses indicate the incorporation of a plurality of bases, leading to multiple oligonucleotides in the primer preparation. In other words, nucleotide residues in parentheses of the aforementioned DNAs were incorporated in the presence of a mixture of plural bases at the time of synthesis, provided that I denotes inosine.

The aforementioned abbreviations in parentheses are reference numbers indicated when the GenBank/EMBL data base is retrieved and are usually called "Accession Numbers".

Example 16

Cloning of Rabbit Gastropyrolic Part Smooth Muscle-Derived G Protein Coupled Receptor Protein cDNA

(1) Preparation of Poly(A)⁺ RNA Fraction from Rabbit

Gastropyrolic Part Smooth Muscle and Synthesis of cDNA

A total RNA was prepared from rabbit gastropyrolic part smooth muscles by the guanidine thiocyanate method (Kaplan B.B. et al., Biochem. J. 183, 181-184 (1979)) and, then, poly(A)⁺ RNA fractions were prepared with a mRNA purifying kit (Pharmacia Co.). Next, to 5 μ g of the

poly(A)⁺ RNA fraction was added a random DNA hexamer (BRL Co.) as a primer, and the resulting mixture was subjected to reaction with MMLV reverse transcriptase (BRL Co.) in the buffer attached to the MMLV reverse transcriptase kit to synthesize complementary DNAs. The reaction product was extracted with phenol/chloroform (1:1), precipitated in ethanol, and was then dissolved in 30 μ l of TE.

(2) Amplification of Receptor cDNA by PCR Using Rabbit Gastropyrolic Part Smooth Muscle-Derived cDNA and Sequencing

By using, as a template, 1 μ l of cDNA prepared from the rabbit gastropyrolic part smooth muscle in the above step (1), PCR amplification using the DNA primer having a nucleotide sequence represented by SEQ ID NO: 10 and the DNA primer having a nucleotide sequence represented by SEQ ID NO: 11 synthesized in Example 15 was carried out. A reaction solution was composed of the synthetic DNA primers (SEQ: 5' primer sequence and 3' primer sequence) each in an amount of 100 pM, 0.25 mM dNTPs, 1 μ l of Taq DNA polymerase and 10 μ l of buffer attached to the enzyme kit, and the total amount of the reaction solution was made to be 100 μ l. The cycle for amplification including 96 °C for 30 sec., 45 °C for 1 min. and 60 °C for 3 min. was repeated 25 times with a Thermal Cycler (Perkin-Elmer Co.). The amplified products were confirmed relying upon 1.2% agarose gel electrophoresis and ethidium bromide staining.

(3) Subcloning of PCR Product into Plasmid Vector and Selection of Novel Receptor Candidate Clone via Decoding Nucleotide Sequence of Inserted cDNA Region

The PCR products obtained in the above step (2) were separated with a 1.4% low-melting temperature agarose gel, the band parts were excised from the gel with a razor blade, and were eluted electrophoretically, extracted with phenol and precipitated in ethanol to recover DNAs. According to the protocol attached to a TA Cloning Kit (Invitrogen Co.),

the recovered DNAs were subcloned to the plasmid vector, pCRTM II. The recombinant vectors were introduced into E. coli JM109 competent cells (Takara Shuzo Co., Japan) to produce transformants. Then, transformant clones having

5 a cDNA-inserted fragment were selected in an LB agar culture medium containing ampicillin, IPTG and X-gal. Only transformant clones exhibiting white color were picked with a sterilized toothstick to obtain 100 transformant clones.

The individual clones were cultured overnight in an

10 LB culture medium containing ampicillin and treated with the automatic plasmid extracting machine PI-100 (Kurabo Co., Japan) to prepare plasmid DNAs. An aliquot of the DNA thus prepared was cut by EcoRI to confirm the size of the cDNA fragment that was inserted. An aliquot of the remaining DNA was

15 further processed with RNase, extracted with phenol/chloroform, and precipitated in ethanol so as to be condensed. Sequencing was carried out by using a DyeDeoxy terminator cycle sequencing kit (ABI Co.), the DNAs were decoded by using a fluorescent automatic sequencer.

20 Homology retrieval was carried out based upon the determined nucleotide sequence by using DNASIS (Hitachi System Engineering Co., Japan). As a result, it was learned that a novel G protein coupled receptor protein was encoded by the cDNA fragment insert in the plasmid possessed by

25 the transformant Escherichia coli JM109/pMN7. Figure 56 and Figure 56 show the nucleotide sequences of the cDNA fragments. To further confirm this fact, by using DNASIS (Hitachi System Engineering Co., Japan), the nucleotide sequences were converted into amino acid sequences [Figure 55] and [Figure 56],

30 and hydrophobicity plotting was carried out [Figure 57]. As a result, the presence of hydrophobic domains which prove that it is a G protein coupled receptor protein were confirmed. Furthermore, homology retrieval was carried out at the amino acid sequence level to find that the DNAs were novel receptor

35 proteins having 27% homology relative to rat-derived β_3 -adrenaline receptor protein (A41679), 29% homology relative to rat-derived serotonin (5-HT6) receptor protein (JN0591),

27% homology relative to dog-derived histamine H₂ receptor protein (A39008), 27% homology relative to human-derived somatostatin receptor (type 4) protein (JN0605), 24% homology relative to human-derived dopamine D₁ receptor protein (S11377), 23% homology relative to rat-derived neurotensin receptor protein (JH0164), 31% homology relative to human-derived cholecystokinin B receptor protein (JC1352), and 30% homology relative to rat-derived gastrin receptor protein (JQ1614). The aforementioned abbreviations in parentheses are reference numbers assigned when they are registered as data to NBRF-PIR and are usually called "Accession Numbers".

Example 17

Amplification of Receptor cDNA by PCR Using MIN6-Derived cDNA and Sequencing

By using, as a template, 5 μ l of cDNA prepared from the mouse pancreatic β -cell strain, MIN6 in Working Example 4 (1), PCR amplification using the DNA primers synthesized in Example 4 (2) as disclosed in Libert F. et al., "Science, 244:569-572, 1989", i.e., a synthetic primer represented by the following sequence:

5'-CTGTG (C or T) G (C or T) (G or C) AT (C or T) GCIIT
(G or T) GA (C or T) (A or C) G (G or C) TAC-3'

(SEQ ID NO: 60)

wherein I is inosine; and
a synthetic primer represented by the following sequence:

5'-A (G or T) G (A or T) AG (A or T) AGGGCAGCCAGCAGAI
(G or C) (A or G) (C or T) GAA-3'

(SEQ ID NO: 61)

wherein I is inosine, was carried out under the same conditions as in Example 3 (1). The resulting PCR product was subcloned to the plasmid vector, pCRTM II, in the same manner as in Example 3 (2) to obtain a plasmid, p5S38. The plasmid p5S38 was transfected into E. coli JM109 to obtain

transformant Escherichia coli JM109/p5S38.

5 The reaction for determining the nucleotide sequence (sequencing) was carried out with a DyeDeoxy terminator cycle sequencing kit (ABI Co.), the DNA was decoded with the fluorescent automatic sequencer (ABI Co.), and the data of the nucleotide sequence obtained were read with DNASIS (Hitachi System Engineering Co., Japan).

10 Figure 62 shows a mouse pancreatic β -cell strain MIN6-derived G protein coupled receptor protein-encoding DNA (SEQ ID NO: 33) and an amino acid sequence (SEQ ID NO: 28) encoded by the isolated DNA based upon the nucleotide sequence of plasmid, p5S38. The underlined portions represent regions corresponding to the synthetic primers.

15 Homology retrieval was carried out based upon the determined nucleotide sequence [Figure 62]. As a result, it was learned that a novel G protein coupled receptor protein was encoded by the cDNA fragment obtained. To further confirm this fact, by using DNASIS (Hitachi System Engineering Co., Japan), the nucleotide sequence was converted into an
20 amino acid sequence [Figure 62], and hydrophobicity plotting was carried out to confirm the presence of four hydrophobic regions [Figure 64]. Upon comparing the amino acid sequence with those encoded by p19P2 obtained in Example 3 (2) and encoded by pG3-2 obtained in Example 4 (2), furthermore,
25 a high degree of homology was found as shown in Figure 63. As a result, it is strongly suggested that the mouse pancreatic β -cell strain, MIN6-derived G protein coupled receptor protein encoded by p5S38 recognizes the same ligand as the human pituitary gland-derived G protein coupled receptor
30 protein encoded by p19P2 does while the animal species from which the receptor protein encoded by p5S38 is derived is different from that from which the receptor protein encoded by p19P2 is. It is also strongly suggested that the mouse pancreatic β -cell strain, MIN6-derived G protein coupled
35 receptor protein encoded by p5S38 recognizes the same ligand as the mouse pancreatic β -cell strain, MIN6-derived G protein coupled receptor proteins encoded by pG3-2 and pG1-10 do and

they are analogous receptor proteins one another (so-called "subtype").

Example 18

Northern Hybridization with cDNA Fragment Included in MIN6-Derived Receptor Protein-Encoding p3H2-17

Mouse cell line, MIN6, Neuro-2a, poly(A)⁺RNA (2.5 μg) and mouse brain, spleen, thymus and pancreas poly(A)⁺RNAs (2.5 μg) were used as poly(A) RNAs. The DNA fragment inserted into the plasmid, p3H2-17, obtained in Example 7 (3) was recovered as a DNA fragment with about 400bp by cutting the plasmid with EcoRI and the resulting DNA fragment was labeled by incorporation of [³²P]dCTP, (Dupont Co.) with a random prime DNA labeling kit (Amasham Co.). The about 400bp labeled DNA fragment was used as a probe for hybridization.

Nylon membrane (PaLL Biodyne, U.S.A.) was used as a filter for northern blotting and migration of the poly(A)⁺RNA and adsorption (sucking) thereof with the blotting filter was carried out according to the method as disclosed in Molecular Cloning, Cold Spring Harbor Laboratory Press, 1989.

The hybridization was carried out by incubating the above-mentioned filter and probe in a buffer containing 50% formamide, 5 x SSPE (20 x SSPE (pH 7.4) is 3 M NaCl, 0.2 M NaH₂PO₄·H₂O, 25 mM EDTA), 5 X Denhardt's solution (Nippon Gene, Japan), 0.1% SDS and 100 μg/ml of salmon sperm DNA overnight at 42 °C. The filter was washed with 0.1 x SSC (20 x SSC is 3 M NaCl, 0.3 M sodium citrate), 0.1% SDS at 50 °C and, after drying with an air, was exposed to an X-ray film (XAR5, Kodak) for 15 days at -80 °C. The results were as shown in Figure 65.

It is considered from Figure 65 that mRNA for the the receptor gene encoded by the cDNA fragment included in p3H2-17 is expressed in the cell line, MIN6, Neuro-2a, and the mouse brain, pancreas, spleen and thymus and especially expressed in the mouse spleen and thymus intensely. The MIN6 signal position hybridizable in the northern hybridization plotting is different from those of other organ cells.

Example 19

PCR Cloning of cDNA Comprising Whole Coding Regions of
Receptor Proteins from Mouse Spleen, Thymus-Derived
Poly(A)⁺ RNA and Sequencing

5 (1) PCR Cloning of cDNA Comprising Whole Coding Region of
Receptor Protein

In order to obtain a full-length open reading frame
(coding region) of the receptor protein encoded by the cDNA
fragment included in p3H2-17, PCR amplification was carried out
10 by 5'RACE and 3'RACE wherein poly(A)⁺ RNA derived from mouse
spleen and thymus was used.

Based on the nucleotide sequence of 3H2-17 which was
disclosed, the following 4 primers were synthesized:

(Nucleotide sequence of synthesized primer)

- 15 ① 5'-TAGTGTGTGGAGTCGTGTGGCTGGCTG-3' (SEQ ID NO: 20)
- ② 5'-AGTCTTTGCTGCCACAGGCATCCAGCG-3' (SEQ ID NO: 21)
- ③ 5'-CAAGCCAGTAAGGCTATGAAGGGCAGCAAG-3' (SEQ ID NO: 22)
- 20 ④ 5'-ACAGGACCTGCTGGGCCATCCTGGCGACACA-3' (SEQ ID NO: 23)

The 5'RACE was carried out according to the protocol
of 5'Ampli Finder RACE kit from ClonTech Co. (ClonTech Co.).

25 In an embodiment, cDNA was prepared from 2 μ g each
of poly(A)⁺ RNAs derived from mouse spleen and thymus by using
the aforementioned primer ④ and ligated with an anchor
attached to the 5'RACE kit. A mixture of a 1/200 amount of the
cDNA thus prepared, the anchor and the aforementioned primer
30 ③ was subjected to PCR using 4 polymerases, Taq (Takara, Japan),
Ex Taq (Takara, Japan), Vent (New England Biolabs) and Pfu
(Stratagene) under the following conditions: 96 °C for 30 sec.,
60 °C for 60 sec., 72 °C for 90 sec. and 35 cycles. A 1/5
amount of the PCR product was subjected to agarose
35 electrophoresis and stained with ethidium bromide (EtBr).
The results are shown in Figure 66. The amplified DNA band

appeared at an about 1 kbp position and the isolated about
1 kbp DNA band which was synthesized from poly(A)⁺ RNAs
derived from mouse spleen and thymus by the 5'RACE using
Ex Taq polymerase was treated with SUPRECTM-01 (Takara, Japan)
5 to recover cDNA.

The isolated DNA was subcloned into pCRTMII vector
by using a TA Cloning Kit (Invitrogen Co.) and the vector was
transfected into E. coli JM109 to obtain 3 transformant
clones, N26, N64 and N75. The clone, N26, holds the thymus-
10 derived cDNA which is amplified by the 5'RACE and the clone,
N75, holds the spleen-derived cDNA which is amplified by the
5'RACE (Figure 68).

The 3'RACE was carried out according to the protocol
of 3' RACE kit (GIBCO BRL Co.).

15 In an embodiment, cDNA was prepared from 1 μ g each
of poly(A)⁺ RNAs derived from mouse spleen and thymus by using
an adaptor primer attached to the 3' RACE kit. A mixture of
the adaptor primer thus prepared and a 1/10 amount of cDNA
which was prepared by using the aforementioned primer ①
20 was subjected to 1st PCR using 4 polymerases, Taq (Takara,
Japan), Ex Taq (Takara, Japan), Vent (NEB) and Pfu (Stratagene)
under the following conditions: 96 °C for 30 sec., 55 °C for 60
sec., 72 °C for 120 sec. and 30 cycles. A mixture of a 1/50
amount of the 1st PCR product, the aforementioned primer ②
25 and the adaptor primer was subjected to 2nd PCR using the
aforementioned polymerases under the same conditions as
aforementioned herein in the 5'RACE process. A 1/5 amount of
the 2nd PCR product was subjected to agarose electrophoresis
and stained with ethidium bromide. The results are shown in
30 Figure 67.

The amplified DNA band appeared at an about 1 kbp
position (which was synthesized from poly(A)⁺ RNAs derived from
mouse thymus by the 3'RACE using Vent polymerase) and the
amplified DNA band appeared at an about 1 kbp position (which
35 was synthesized from poly(A)⁺ RNAs derived from mouse thymus
by the 3'RACE using Pfu polymerase) were treated with

SUPRECTM-01 (Takara, Japan) to recover cDNA, respectively.

The isolated DNAs were treated with T4 polynucleotide kinase (Wako Pure Chemical Co., Japan) to add phosphate to the end thereof and the phosphorylated DNAs were ligated with pUC18 SmaI BAP (Pharmacia) by using DNA Ligation Kit (Takara, Japan) followed by transformation of *E. coli* JM109 to obtain 3 transformant clones, C2, C13 and C15. The clones, C13 and C15, hold the thymus-derived cDNA which is amplified by the 3'RACE and the clone, C2, holds the thymus-derived cDNA which is amplified by the 3'RACE (Figure 68).

Based on the nucleotide sequences of clones, N26, N64 and N75, which are considered to hold the N-terminal region of the open reading frame (ORF) of the cDNA fragment included in p3H2-17 and the nucleotide sequences of clones, C2, C13 and C15, which are considered to hold the C-terminal region of the open reading frame (ORF) of the cDNA fragment included in p3H2-17, the entire nucleotide sequence coding for the open reading frame and neighboring region of the receptor protein encoded by the cDNA included in p3H2-17 was determined.

To be more specific, sequencing was carried out with the primers used in the 5'RACE and 3'RACE or synthetic primers for sequencing by using a DyeDeoxy Terminator Cycle Sequencing Kit (ABI Co.), the nucleotide sequences were decoded by using a fluorescent automatic sequencer. The obtained data of the DNA were analyzed by DNASIS (Hitachi System Engineering Co., Japan).

PCR errors which presumably happen to occur upon PCR have been corrected by a way of thinking that, when nucleotides between two clones which are independently produced by PCR are identical (e.g. those between clones, N75 and N64, are identical) each other, the identical base is considered as correct. The determined nucleotide sequence is shown in Figure 69. The amino acid sequence is deduced based on the determined nucleotide sequence (Figure 69). Hydrophobicity plotting was carried out based on the deduced amino acid sequence (Figure 70). As a result, it was learned that it was a seven transmembrane G protein coupled receptor, as

it is suggested from the cDNA fragment included in p3H2-17.

Homology retrieval at the amino acid level indicates that it is homologous to mouse P_{2U} purinoceptor and chicken P_{2Y} purinoceptor.

5 Further, the clone which are free of an error in the open reading frame (ORF) was selected and used to construct plasmids carrying the full-length ORF of the receptor protein encoded by p3H2-17. In an embodiment, the cDNA fragment held by the clone, N75, was digested with restriction enzymes,
10 DraIII and EcoRI, to obtain cDNA fragments which are the N-terminal region of the receptor protein held by p3H2-17. The C-terminal cDNA fragment encoded by C13 was digested with restriction enzymes, DraIII and EcoRI, to delete 5'-side regions from the DraIII site of the C-terminal and the long
15 fragment was obtained by the digestion of C13 with restriction enzymes, DraIII and EcoRI. The N75-derived N-terminal cDNA DraIII-EcoRI fragment was ligated with the long C13-derived DraIII-EcoRI fragment by using a DNA Ligation Kit (Takara, Japan) and transfected into Escherichia coli JM109 to obtain
20 transformant Escherichia coli JM109/pMAH2-17.

(2) Electrophysiological Measurement of Receptor Encoded by pMAH2-17

The receptor encoded by pMAH2-17 was examined electrophysiologically in Xenopus oocytes. The ORF of the
25 receptor encoded by pMAH2-17 was inserted into the XhoI-XbaI sites of pBluescriptTM II SK(+) (Stratagene) with directing the sequence thereof downstream from T7 promoter. The resulting plasmid as a template was treated with a mCAPTM mRNA Capping kit (Stratagene) to produce cRNA of this receptor
30 gene.

The cRNA was injected into Xenopus oocytes (50ng cRNA/50nl/oocyte), previously prepared according to the method disclosed in Nathan Dascal et al., Proc. Natl. Acad. Sci. USA, Vol. 90, pp.6596-6600 (1993). The cRNA-injected oocytes were
35 incubated at 20 °C for 2 to 3 days and subjected to electrophysiological measurements. The measurement was carried

out with a microelectrode-applicable high input resistance amplifier (MEz-8300, Nippon Kodan, Co., Japan), and a voltage clamping amplifier (CEz -/200, Nippon Kodan, Co., Japan). The initial membrane potential of oocytes was set to -60 mV and responses (current changes of the membrane) evoked by addition of ligands were recorded with a recorder (Thermal Array recorder, Nippon Kodan, Co., Japan) (Nathan Dascal et al., Proc. Natl. Acad. Sci. USA, Vol. 90, pp.6596-6600 (1993)).

Typical inward currents elicited upon activation of phospholipase C-coupled receptors were observed in oocytes injected with pMAH2-17 cRNA via stimulation by 10 μ M ATP (Figure 75). In contrast, such a current was not observed in oocytes injected with H₂O, instead of pMAH2-17 cRNA, by the ATP stimulation.

In conclusion, it is considered that the receptor encoded by pMAH2-17 cRNA is classified into a subtype within the ATP receptor, P₂ purinoceptor.

Example 20

Cloning of Rabbit Gastropyrolic Part Smooth Muscle-Derived G Protein Coupled Receptor Protein cDNA

(1) Preparation of Poly(A)⁺ RNA Fraction from Rabbit

Gastropyrolic Part Smooth Muscle and Synthesis of cDNA

A total RNA was prepared from rabbit gastropyrolic part smooth muscles by the guanidine thiocyanate method (Kaplan B.B. et al., Biochem. J. 183, 181-184 (1979)) and, then, poly(A)⁺ RNA fractions were prepared with a mRNA purifying kit (Pharmacia Co.). Next, to 5 μ g of the poly(A)⁺ RNA fraction was added a random DNA hexamer (BRL Co.) as a primer, and the resulting mixture was subjected to reaction with MMLV reverse transcriptase (BRL Co.) in the buffer attached to the MMLV reverse transcriptase kit to synthesize complementary DNAs. The reaction product was extracted with phenol/chloroform (1:1), precipitated in ethanol, and was then dissolved in 30 μ l of TE.

(2) Amplification of Receptor cDNA by PCR Using Rabbit
Gastropyrolic Part Smooth Muscle-Derived cDNA and
Sequencing

5 By using, as a template, 1 μ l of cDNA prepared
from the rabbit gastropyrolic part smooth muscle in the above
step (1), PCR amplification using the DNA primer having a
nucleotide sequence represented by SEQ ID NO: 10 and the DNA
primer having a nucleotide sequence represented by SEQ ID
NO: 4 synthesized in Example 15 was carried out.

10 A reaction solution was composed of the synthetic DNA primers
(SEQ: 5' primer sequence and 3' primer sequence) each in an
amount of 100 pM, 0.25 mM dNTPs, 1 μ l of Taq DNA polymerase
and 10 μ l of buffer attached to the enzyme kit, and the
total amount of the reaction solution was made to be 100 μ l.
15 The cycle for amplification including 96 °C for 30 sec., 45 °C
for 1 min. and 60 °C for 3 min. was repeated 25 times by using
a Thermal Cycler (Perkin-Elmer Co.). The amplified products
were confirmed relying upon 1.2% agarose gel electrophoresis
and ethidium bromide staining.

20 (3) Subcloning of PCR Product into Plasmid Vector and
Selection of Novel Receptor Candidate Clone via Decoding
Nucleotide Sequence of Inserted cDNA Region

The PCR products obtained in the above step (2)
were separated by using a 1.0% low-melting temperature agarose
25 gel, the band parts were excised from the gel with a razor
blade, and were electro-eluted, extracted with phenol and
precipitated in ethanol to recover DNAs. According to the
protocol attached to a TA Cloning Kit (Invitrogen Co.),
the recovered DNAs were subcloned to the plasmid vector,
30 pCRTM II. The recombinant vectors were introduced into
E. coli JM109 competent cells (Takara Shuzo Co., Japan) to
produce transformants. Then, transformant clones having
a cDNA-inserted fragment were selected in an LB agar
culture medium containing ampicillin, IPTG and X-gal. Only
35 transformant clones exhibiting white color were picked with
a sterilized toothstick to obtain 100 transformant clones.

The individual clones were cultured overnight in an LB culture medium containing ampicillin and treated with the automatic plasmid extracting machine PI-100 (Kurabo Co., Japan) to prepare plasmid DNAs. An aliquot of the DNAs thus prepared was cut by EcoRI to confirm the size of the cDNA fragment that was inserted. An aliquot of the remaining DNAs was further processed with RNase, extracted with phenol/chloroform, and precipitated in ethanol so as to be condensed. Sequencing was carried out by using a DyeDeoxy terminator cycle sequencing kit (ABI Co.), the DNAs were decoded by using a fluorescent automatic sequencer.

Homology retrieval was carried out based upon the determined nucleotide sequence. As a result, it was learned that a novel G protein coupled receptor protein was been encoded by the cDNA fragment insert in the plasmid possessed by the transformant Escherichia coli JM109/pMN128. The nucleotide sequences of the cDNA fragments are shown in Figures 71 and 72. To further confirm this fact, by using DNASIS (Hitachi System Engineering Co., Japan) the nucleotide sequences were converted into amino acid sequences [Figure 71 and Figure 72], and homology retrieval was carried out in view of hydrophobicity plotting [Figure 73] and at the amino acid sequence level to find a novel receptor protein which has 27% homology relative to hamster-derived β_2 -adrenaline receptor protein (A03159), 20% homology relative to rat-derived bradykinin receptor (type B_2) protein (A41283), 24% homology relative to human-derived dopamine D_1 receptor protein (S11377) and 23% homology relative to human-derived blue sensitive opsin receptor protein (A03156). The aforementioned abbreviations in parentheses are reference numbers assigned when they are registered as data to NBRF-PIR and are usually called "Accession Numbers".

Cloning of cDNA Comprising Whole Coding Regions for Receptor Protein from Human-Derived DNA Library

5

10

15

② 5'-ACAGGACCTGCTGGGCCATCCTGGCGACACA-3'

(SEQ ID NO: 23)

20

25

30

③ 5'-ACAGCCATCTTCGCTGCCACAGGCAT-3'

④ 5'-AGACAGTAGCAGGCCAGCAGGGCAGCAAA-3'

(SEO ID NO: 59)

The above synthetic 2 primers were each used in combination with λ gt 11 primers (Takara, Japan; catalogue 3864) for obtaining full-length human purinoceptor cDNA. Thus, using thermally denatured, human placenta-derived λ gt 11 cDNA libraries (CLONTECH; CLHL 1008b), first RCR amplification using a combination of the DNA primer having a nucleotide sequence represented by SEQ ID NO: 20 with λ gt 11 Forward primer, of the DNA primer having a nucleotide sequence represented by SEQ ID NO: 20 with λ gt 11 Reverse primer, of the DNA primer having a nucleotide sequence represented by SEQ ID NO: 23 with λ gt 11 Forward primer, and of the DNA primer having a nucleotide sequence represented by SEQ ID NO: 23 with λ gt 11 Reverse primer was carried out with Ex Taq polymerase (Takara, Japan) (30 cycles; 95°C/30 seconds, 55 °C/60 seconds, and 72 °C/60 seconds), respectively.

Next, by using a 1/50 of the 1st PCR product, second RCR amplification was carried in the same manner as in the first PCR except for using the DNA primer having a nucleotide sequence represented by SEQ ID NO: 58 in place of SEQ ID NO: 20 and the DNA primer having a nucleotide sequence represented by SEQ ID NO: 59 in place of SEQ ID NO: 23 (30 cycles; 95°C/30 seconds, 65 °C/60 seconds and 72 °C/60 seconds). The amplified product DNA was subcloned using a TA Cloning Kit (Invitrogen Co.) and sequencing was carried out for three clones each of 5' and 3' sides (Figure 77).

Based on the amino acid sequence (Figure 77) deduced from the determined nucleotide sequence of human purinoceptor cDNA as shown in Figure 77, hydrophobicity plotting was carried out (Figure 78). As a result, it was learned that the human-derived receptor is a novel seven transmembrane G protein coupled receptor, similarly to the mouse type. It was also learned that the deduced amino acid sequence of human receptor has 87% homology relative to the amino acid sequence of mouse purinoceptor and its amino acid residues are well conserved (Figure 79).

Clones free of PCR errors which often occur in a PCR amplification were selected and restriction enzyme regions

comprising overlapping areas were obtained therefrom.

The restriction enzyme regions thus obtained were subjected to construction of plasmid pAH2-17 having a full-length open reading frame of human purinoceptor cDNA. The plasmid pAH2-17 is possessed by transformant Escherichia coli JM109/pAH2-17.

The DNA primers of the present invention allow efficient amplification of DNAs that encode G protein coupled receptor proteins. This makes it possible to efficiently screen for the DNAs coding for G protein coupled receptor proteins and to accomplish the cloning.

The G protein coupled receptor protein of the present invention and their G protein coupled receptor protein-encoding DNA are advantageously useful in:

- ① determining ligands,
- ② obtaining antibodies and an antisera,
- ③ constructing systems for expressing recombinant receptor proteins,
- ④ investigating or developing receptor-binding assay systems and screening for pharmaceutical candidate compounds, by using the above expression system
- ⑤ designing drugs based upon comparisons with ligands and receptors having a structure similar or analogous thereto,
- ⑥ preparing probes and/or PCR primers in gene diagnosis, and
- ⑦ gene manipulating therapy.

In particular, discovering the structure and properties of the G protein coupled receptor will lead to the development of unique pharmaceuticals acting upon these systems.

The practice of the present invention will employ, otherwise indicated, conventional techniques of molecular biology, microbiology, recombinant DNA, pharmacology, immunology, bioscience, and medical technology, which are within the skill of the art. All patents, patent applications, and publications mentioned herein, both supra and infra, are hereby incorporated herein by reference.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: Takeda Chemical Industries, Ltd.
(B) STREET: 1-1, Doshomachi 4-chome, Chuo-ku
(C) CITY: Osaka-shi
(D) STATE: Osaka
(E) COUNTRY: Japan
(F) POSTAL CODE (ZIP): 541

(ii) TITLE OF INVENTION: G Protein Coupled Receptor Protein,
Production, And Use Thereof

(iii) NUMBER OF SEQUENCES: 61

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(iii) FEATURES: N is A, G, C, or T

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

CGTGGSCMTS STGGGCAACN YCCTG 25

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(iii) FEATURES: N is A, G, C, or T

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

GTNGWRRGGC ANCCAGCAGA KGGCAA 27

(2) INFORMATION FOR SEQ ID NO: 3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 27
 - (B) TYPE: Nucleic acid
 - (C) STRANDEDNESS: Single
 - (D) TOPOLOGY: Linear
- (ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA
- (iii) FEATURES: N is inosine
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

CTCGCSGCM TNRGYATGGA YCGNTAT 27

(2) INFORMATION FOR SEQ ID NO: 4:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30
 - (B) TYPE: Nucleic acid
 - (C) STRANDEDNESS: Single
 - (D) TOPOLOGY: Linear
- (ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA
- (iii) FEATURES: N is inosine
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

CATGTRGWAG GGAANCCAGS AMANRARRAA 30

(2) INFORMATION FOR SEQ ID NO: 5:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 27
 - (B) TYPE: Nucleic acid
 - (C) STRANDEDNESS: Single
 - (D) TOPOLOGY: Linear
- (ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA
- (iii) FEATURES: N is inosine
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

CTGACYGYTC TNRSNRYTGA CMGVTAC 27

(2) INFORMATION FOR SEQ ID NO: 6:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 27
 - (B) TYPE: Nucleic acid
 - (C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(iii) FEATURES: N is inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

CTGACYGYTC TNRSNRYTGA CMGVTAT 27

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(iii) FEATURES: N is inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

CTCGCSGCMY TNRGYATGGA YCGNTAC 27

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(iii) FEATURES: N is inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

GATGTGRTAR GGSRNCCAAC AGANGRYAAA 30

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(iii) FEATURES: N is inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

GATGTGRTAR GGSRNCCAAC AGANGRYGAA 30

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(iii) FEATURES: N is inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GYCACCAACN WSTTCATCCT SWNHCTG 27

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(iii) FEATURES: N is inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

ASNSANRAAG SARTAGANGA NRGGRTT 27

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(iii) FEATURES: N is inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

TGNTSSTKMT NGSNGTKGTN GGNA 25

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(iii) FEATURES: N is inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

AYCKGTAYCK GTCCANKGWN ATKGC 25

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(iii) FEATURES: N is inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

CATKKCCSTG GASAGNTAYN TRGC 24

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(iii) FEATURES: N is inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

GWGGSAAKC CAGCASANGG CCAA 24

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18

(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(iii) FEATURES: 15th N is A, G, C, or T
6th, 9th, 10th & 12th Ns are inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

ARYYTNGCNN TNGCNGAY

18

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(iii) FEATURES: 13th, 15th, 16th & 18th Ns are
each A, G, C, or T
1st, 4th, 6th Ns are inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

NGGNANCCAR CANANNRNRA A

21

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(iii) FEATURES: N is inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

GCCTSNTNRN SATGWSTGTG GANMGNT

27

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(iii) FEATURES: N is inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

GAWSNTGMYN ANRTGGWAGG GNANCCA 27

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

TAGTGTGTGG AGTCGTGTGG CTGGCTG 27

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

AGTCTTTGCT GCCACAGGCA TCCAGCG 27

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

CAAGCCAGTA AGGCTATGAA GGGCAGCAAG 30

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 31
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

ACAGGACCTG CTGGGCCATC CTGGCGACAC A 31

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 91
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

Leu Val Leu Val Ile Ala Arg Val Arg Arg Leu His Asn Val Thr Asn
1 5 10 15
Phe Leu Ile Gly Asn Leu Ala Leu Ser Asp Val Leu Met Cys Thr Ala
20 25 30
Cys Val Pro Leu Thr Leu Ala Tyr Ala Phe Glu Pro Arg Gly Trp Val
35 40 45
Phe Gly Gly Gly Leu Cys His Leu Val Phe Phe Leu Gln Pro Val Thr
50 55 60
Val Tyr Val Ser Val Phe Thr Leu Thr Thr Ile Ala Val Asp Arg Tyr
65 70 75 80
Val Val Leu Val His Pro Leu Arg Arg Arg Ile
85 90

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 59
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

Gly Leu Leu Leu Val Thr Tyr Leu Leu Pro Leu Leu Val Ile Leu Leu

1	5	10	15
Ser Tyr Val Arg Val Ser Val Lys Leu Arg Asn Arg Val Val Pro Gly	20	25	30
Cys Val Thr Gln Ser Gln Ala Asp Trp Asp Arg Ala Arg Arg Arg Arg	35	40	45
Thr Phe Cys Leu Leu Val Val Val Val Val Val	50	55	

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 370
 (B) TYPE: Amino acid
 (C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

Met Ala Ser Ser Thr Thr Arg Gly Pro Arg Val Ser Asp Leu Phe Ser	1	5	10	15
Gly Leu Pro Pro Ala Val Thr Thr Pro Ala Asn Gln Ser Ala Glu Ala	20	25	30	
Ser Ala Gly Asn Gly Ser Val Ala Gly Ala Asp Ala Pro Ala Val Thr	35	40	45	
Pro Phe Gln Ser Leu Gln Leu Val His Gln Leu Lys Gly Leu Ile Val	50	55	60	
Leu Leu Tyr Ser Val Val Val Val Val Gly Leu Val Gly Asn Cys Leu	65	70	75	80
Leu Val Leu Val Ile Ala Arg Val Arg Arg Leu His Asn Val Thr Asn	85	90	95	
Phe Leu Ile Gly Asn Leu Ala Leu Ser Asp Val Leu Met Cys Thr Ala	100	105	110	
Cys Val Pro Leu Thr Leu Ala Tyr Ala Phe Glu Pro Arg Gly Trp Val	115	120	125	
Phe Gly Gly Gly Leu Cys His Leu Val Phe Phe Leu Gln Pro Val Thr	130	135	140	
Val Tyr Val Ser Val Phe Thr Leu Thr Thr Ile Ala Val Asp Arg Tyr	145	150	155	160
Val Val Leu Val His Pro Leu Arg Arg Arg Ile Ser Leu Arg Leu Ser	165	170	175	
Ala Tyr Ala Val Leu Ala Ile Trp Ala Leu Ser Ala Val Leu Ala Leu				

	180		185		190										
Pro	Ala	Ala	Val	His	Thr	Tyr	His	Val	Glu	Leu	Lys	Pro	His	Asp	Val
	195						200					205			
Arg	Leu	Cys	Glu	Glu	Phe	Trp	Gly	Ser	Gln	Glu	Arg	Gln	Arg	Gln	Leu
	210					215					220				
Tyr	Ala	Trp	Gly	Leu	Leu	Leu	Val	Thr	Tyr	Leu	Leu	Pro	Leu	Leu	Val
	225				230					235					240
Ile	Leu	Leu	Ser	Tyr	Val	Arg	Val	Ser	Val	Lys	Leu	Arg	Asn	Arg	Val
				245					250					255	
Val	Pro	Gly	Cys	Val	Thr	Gln	Ser	Gln	Ala	Asp	Trp	Asp	Arg	Ala	Arg
			260					265					270		
Arg	Arg	Arg	Thr	Phe	Cys	Leu	Leu	Val	Val	Val	Val	Val	Val	Phe	Ala
			275				280						285		
Val	Cys	Trp	Leu	Pro	Leu	His	Val	Phe	Asn	Leu	Leu	Arg	Asp	Leu	Asp
	290					295					300				
Pro	His	Ala	Ile	Asp	Pro	Tyr	Ala	Phe	Gly	Leu	Val	Gln	Leu	Leu	Cys
	305				310					315					320
His	Trp	Leu	Ala	Met	Ser	Ser	Ala	Cys	Tyr	Asn	Pro	Phe	Ile	Tyr	Ala
				325					330					335	
Trp	Leu	His	Asp	Ser	Phe	Arg	Glu	Glu	Leu	Arg	Lys	Leu	Leu	Val	Ala
			340					345					350		
Trp	Pro	Arg	Lys	Ile	Ala	Pro	His	Gly	Gln	Asn	Met	Thr	Val	Ser	Val
		355				360						365			
Val	Ile														
	370														

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 206
 (B) TYPE: Amino acid
 (C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

Leu	Val	Leu	Val	Ile	Ala	Arg	Val	Arg	Arg	Leu	Tyr	Asn	Val	Thr	Asn
1				5				10						15	
Phe	Leu	Ile	Gly	Asn	Leu	Ala	Leu	Ser	Asp	Val	Leu	Met	Cys	Thr	Ala
			20					25					30		
Cys	Val	Pro	Leu	Thr	Leu	Ala	Tyr	Ala	Phe	Glu	Pro	Arg	Gly	Trp	Val

35	40	45
Phe Gly Gly Gly Leu Cys His Leu Val Phe Phe Leu Gln Ala Val Thr		
50	55	60
Val Tyr Val Ser Val Phe Thr Leu Thr Thr Ile Ala Val Asp Arg Tyr		
65	70	75
Val Val Leu Val His Pro Leu Arg Arg Arg Ile Ser Leu Arg Leu Ser		
85	90	95
Ala Tyr Ala Val Leu Ala Ile Trp Val Leu Ser Ala Val Leu Ala Leu		
100	105	110
Pro Ala Ala Val His Thr Tyr His Val Glu Leu Lys Pro His Asp Val		
115	120	125
Arg Leu Cys Glu Glu Phe Trp Gly Ser Gln Glu Arg Gln Arg Gln Leu		
130	135	140
Tyr Ala Trp Gly Leu Leu Leu Val Thr Tyr Leu Leu Pro Leu Leu Val		
145	150	155
Ile Leu Leu Ser Tyr Ala Arg Val Ser Val Lys Leu Arg Asn Arg Val		
165	170	175
Val Pro Gly Arg Val Thr Gln Ser Gln Ala Asp Trp Asp Arg Ala Arg		
180	185	190
Arg Arg Arg Thr Phe Cys Leu Leu Val Val Val Val Val Val		
195	200	205

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 126
- (B) TYPE: Amino acid
- (C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

Val Val Leu Val His Pro Leu Arg Arg Arg Ile Ser Leu Arg Leu Ser
1 5 10 15
Ala Tyr Ala Val Leu Gly Ile Trp Ala Leu Ser Ala Val Leu Ala Leu
20 25 30
Pro Ala Ala Val His Thr Tyr His Val Glu Leu Lys Pro His Asp Val
35 40 45
Ser Leu Cys Glu Glu Phe Trp Gly Ser Gln Glu Arg Gln Arg Gln Ile
50 55 60
Tyr Ala Trp Gly Leu Leu Leu Gly Thr Tyr Leu Leu Pro Leu Leu Ala

65		70		75		80									
Ile	Leu	Leu	Ser	Tyr	Val	Arg	Val	Ser	Val	Lys	Leu	Arg	Asn	Arg	Val
				85					90					95	
Val	Pro	Gly	Ser	Val	Thr	Gln	Ser	Gln	Ala	Asp	Trp	Asp	Arg	Ala	Arg
			100					105					110		
Arg	Arg	Arg	Thr	Phe	Cys	Leu	Leu	Val	Val	Val	Val	Val	Val	Val	Val
			115				120						125		

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 273
 (B) TYPE: Nucleic acid
 (C) STRANDEDNESS: Double
 (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE

(C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

CTGGTGCTGG	TGATCGCGCG	GGTGCGCCGG	CTGCACAACG	TGACGAACTT	CCTCATCGGC	60
AACCTGGCCT	TGTCCGACGT	GCTCATGTGC	ACCGCCTGCG	TGCCGCTCAC	GCTGGCCTAT	120
GCCTTCGAGC	CACGCGGCTG	GGTGTTCCGC	GGCGGCCTGT	GCCACCTGGT	CTTCTTCCTG	180
CAGCCGGTCA	CCGTCTATGT	GTCGGTGTTT	ACGCTCACCA	CCATCGCAGT	GGACCGGTAC	240
GTCGTGCTGG	TGCACCCGCT	GAGGCGGCGC	ATC			273

(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 177
 (B) TYPE: Nucleic acid
 (C) STRANDEDNESS: Double
 (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE

(C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

GGCCTGCTGC	TGGTCACCTA	CCTGCTCCCT	CTGCTGGTCA	TCCTCCTGTC	TTACGTCCGG	60
GTGTCAGTGA	AGCTCCGCAA	CCGCGTGGTG	CCGGGCTGCG	TGACCCAGAG	CCAGGCCGAC	120
TGGGACCGCG	CTCGGCGCCG	GCGCACCTTC	TGCTTGCTGG	TGGTGGTCGT	GGTGGTG	177

(2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1110
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Double
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE

(C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

ATGGCCTCAT CGACCACTCG GGGCCCCAGG GTTCTGACT TATTTTCTGG GCTGCCGCCG 60
GCGGTCACAA CTCCCGCCAA CCAGAGCGCA GAGGCCTCGG CGGGCAACGG GTCGGTGGCT 120
GGCGCGGACG CTCCAGCCGT CACGCCCTTC CAGAGCCTGC AGCTGGTGCA TCAGCTGAAG 180
GGGCTGATCG TGCTGCTCTA CAGCGTCGTG GTGGTCGTGG GGCTGGTGGG CAACTGCCTG 240
CTGGTGCTGG TGATCGCGCG GGTGCGCCGG CTGCACAACG TGACGAACTT CCTCATCGGC 300
AACCTGGCCT TGTCCGACGT GCTCATGTGC ACCGCCTGCG TGCCGCTCAC GCTGGCCTAT 360
GCCTTCGAGC CACGCGGCTG GGTGTTCCGG GCGGGCCTGT GCCACCTGGT CTTCTTCCTG 420
CAGCCGGTCA CCGTCTATGT GTCGGTGTTT ACGCTACCA CCATCGCAGT GGACCGCTAC 480
GTCGTGCTGG TGCACCCGCT GAGGCGGCGC ATCTCGCTGC GCCTCAGCGC CTACGCTGTG 540
CTGGCCATCT GGGCGCTGTC CGCGGTGCTG GCGCTGCCCC CCGCCGTGCA CACCTATCAC 600
GTGGAGCTCA AGCCGCACGA CGTGCGCCTC TGCAGGAGT TCTGGGGCTC CCAGGAGCGC 660
CAGCGCCAGC TCTACGCCTG GGGGCTGCTG CTGGTCACCT ACCTGCTCCC TCTGCTGGTC 720
ATCCTCCTGT CTTACGTCCG GGTGTCAGTG AAGCTCCGCA ACCGCGTGGT GCCGGGCTGC 780
GTGACCCAGA GCCAGGCCGA CTGGGACCGC GCTCGGCGCC GGCGCACCTT CTGCTTGCTG 840
GTGGTGCTCG TGGTGCTGTT CGCCGTCTGC TGGCTGCCGC TGCACGTCTT CAACCTGCTG 900
CGGGACCTCG ACCCCCACGC CATCGACCCT TACGCCTTTG GGCTGGTGCA GCTGCTCTGC 960
CACTGGCTCG CCATGAGTTC GGCCTGCTAC AACCCCTTCA TCTACGCCTG GCTGCACGAC 1020
AGCTTCCGCG AGGAGCTGCG CAAACTGTTG GTCGCTTGGC CCCGCAAGAT AGCCCCCAT 1080
GGCCAGAATA TGACCGTCAG CGTGGTCATC 1110

(2) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 618
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Double
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE
(C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

CTGGTGCTGG TGATCGCGCG GGTGCGCCGG CTGTACAACG TGACGAATTT CCTCATCGGC	60
AACCTGGCCT TGTCCGACGT GTCATGTGC ACCGCCTGCG TGCCGCTCAC GCTGGCCTAT	120
GCCTTCGAGC CACGCGGCTG GGTGTTTCGGC GGCGGCCTGT GCCACCTGGT CTTCTTCCTG	180
CAGGCGGTCA CCGTCTATGT GTCGGTGTTT ACGCTCACCA CCATCGCAGT GGACCGCTAC	240
GTCGTGCTGG TGCACCCGCT GAGGCGGCGC ATCTCGCTGC GCCTCAGCGC CTACGCTGTG	300
CTGGCCATCT GGGTGCTGTC CGCGGTGCTG GCGCTGCCCC CCGCCGTGCA CACCTATCAC	360
GTGGAGCTCA AGCCGCACGA CGTGCGCCTC TGCGAGGAGT TCTGGGGCTC CCAGGAGCGC	420
CAGCGCCAGC TCTACGCCTG GGGGCTGCTG CTGGTCACCT ACCTGCTCCC TCTGCTGGTC	480
ATCCTCCTGT CTTACGCCCC GGTGTCAGTG AAGCTCCGCA ACCGCGTGGT GCCGGGCCGC	540
GTGACCCAGA GCCAGGCCGA CTGGGACCGC GCTCGGCGCC GGCGCACCTT CTGCTTGCTG	600
GTGGTGGTCG TGGTGGTG	618

(2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 378
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Double
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE
(C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

GTGGTTCTGG TGCACCCGCT ACGTCGGCGC ATTTCACTGA GGCTCAGCGC CTACGCGGTG	60
CTGGGCATCT GGGCTCTATC TGCAGTGCTG GCGCTGCCGG CCGCGGTGCA CACCTACCAT	120
GTGGAGCTCA AGCCCCACGA CGTGAGCCTC TGCGAGGAGT TCTGGGGCTC GCAGGAGCGC	180

CAACGCCAGA TCTACGCCTG GGGGCTGCTT CTGGGCACCT ATTTGCTCCC CCTGCTGGCC 240
 ATCCTCCTGT CTTACGTACG GGTGTCAGTG AAGCTGAGGA ACCGCGTGGT GCCTGGCAGC 300
 GTGACCCAGA GTCAAGCTGA CTGGGACCGA GCGCGTCGCC GCCGCACTTT CTGTCTGCTG 360
 GTGGTGGTGG TGGTAGTG 378

(2) INFORMATION FOR SEQ ID NO: 34:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 70
 (B) TYPE: Amino acid
 (C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

Val Cys His Val Ile Phe Lys Asn Gln Arg Met His Ser Ala Thr Ser
 1 5 10 15
 Leu Phe Ile Val Asn Leu Ala Val Ala Asp Ile Met Ile Thr Leu Ile
 20 25 30
 Asn Thr Pro Phe Thr Leu Val Arg Phe Val Asn Ser Thr Trp Ile Phe
 35 40 45
 Gly Lys Gly Met Cys His Val Ser Arg Phe Ala Gln Tyr Cys Ser Leu
 50 55 60
 His Val Ser Ala Leu Thr
 65 70

(2) INFORMATION FOR SEQ ID NO: 35:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 71
 (B) TYPE: Amino acid
 (C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

Glu Pro Ala Asp Leu Phe Trp Lys Asn Leu Asp Leu Pro Thr Phe Ile
 1 5 10 15
 Leu Leu Asn Ile Leu Pro Leu Leu Ile Ile Ser Val Ala Tyr Val Arg
 20 25 30
 Val Thr Lys Lys Leu Trp Leu Cys Asn Met Ile Val Asp Val Thr Thr
 35 40 45
 Glu Gln Tyr Phe Ala Leu Arg Pro Lys Lys Lys Thr Ile Lys Met
 50 55 60

Leu Met Leu Val Val Val Leu
65 70

(2) INFORMATION FOR SEQ ID NO: 36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 210
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Double
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE
(C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

GTCTGTCATG TCATCTTCAA GAACCAGCGA ATGCACTCGG CCACCAGCCT CTCATCGTC 60
AACCTGGCAG TTGCCGACAT AATGATCACG CTGCTCAACA CCCCTTCAC TTGGTTTCGC 120
TTTGTGAACA GCACATGGAT ATTTGGGAAG GGCATGTGCC ATGTCAGCCG CTTTGCCCAG 180
TACTGCTCAC TGCACGTCTC AGCACTGACA 210

(2) INFORMATION FOR SEQ ID NO: 37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 213
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Double
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE
(C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

GAGCCAGCTG ACCTCTTCTG GAAGAACCTG GACTTGCCCA CCTTCATCCT GCTCAACATC 60
CTGCCCCCTCC TCATCATCTC TGTGGCCTAC GTTCGTGTGA CCAAGAAACT GTGGCTGTGT 120
AATATGATTG TCGATGTGAC CACAGAGCAG TACTTTGCCC TGCGGCCCAA AAAGAAGAAG 180
ACCATCAAGA TGTGATGCT GGTGGTAGTC CTC 213

(2) INFORMATION FOR SEQ ID NO: 38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 115
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

Ala Ser Trp His Lys Arg Gly Gly Arg Arg Ala Ala Trp Val Val Cys
1 5 10 15
Gly Val Val Trp Leu Ala Val Thr Ala Gln Cys Leu Pro Thr Ala Val
20 25 30
Phe Ala Ala Thr Gly Ile Gln Arg Asn Arg Thr Val Cys Tyr Asp Leu
35 40 45
Ser Pro Pro Ile Leu Ser Thr Arg Tyr Leu Pro Tyr Gly Met Ala Leu
50 55 60
Thr Val Ile Gly Phe Leu Leu Pro Phe Ile Ala Leu Leu Ala Cys Tyr
65 70 75 80
Cys Arg Met Ala Arg Arg Leu Cys Arg Gln Asp Gly Pro Ala Gly Pro
85 90 95
Val Ala Gln Glu Arg Arg Ser Lys Ala Ala Arg Met Ala Val Val Val
100 105 110
Ala Ala Val
115

(2) INFORMATION FOR SEQ ID NO: 39:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 328
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

Met Glu Gln Asp Asn Gly Thr Ile Gln Ala Pro Gly Leu Pro Pro Thr
1 5 10 15
Thr Cys Val Tyr Arg Glu Asp Phe Lys Arg Leu Leu Leu Thr Pro Val
20 25 30
Tyr Ser Val Val Leu Val Val Gly Leu Pro Leu Asn Ile Cys Val Ile
35 40 45
Ala Gln Ile Cys Ala Ser Arg Arg Thr Leu Thr Arg Ser Ala Val Tyr
50 55 60
Thr Leu Asn Leu Ala Leu Ala Asp Leu Met Tyr Ala Cys Ser Leu Pro
65 70 75 80

Leu Leu Ile Tyr Asn Tyr Ala Arg Gly Asp His Trp Pro Phe Gly Asp
85 90 95

Leu Ala Cys Arg Phe Val Arg Phe Leu Phe Tyr Ala Asn Leu His Gly
100 105 110

Ser Ile Leu Phe Leu Thr Cys Ile Ser Phe Gln Arg Tyr Leu Gly Ile
115 120 125

Cys His Pro Leu Ala Ser Trp His Lys Arg Gly Gly Arg Arg Ala Ala
130 135 140

Trp Val Val Cys Gly Val Val Trp Leu Ala Val Thr Ala Gln Cys Leu
145 150 155 160

Pro Thr Ala Val Phe Ala Ala Thr Gly Ile Gln Arg Asn Arg Thr Val
165 170 175

Cys Tyr Asp Leu Ser Pro Pro Ile Leu Ser Thr Arg Tyr Leu Pro Tyr
180 185 190

Gly Met Ala Leu Thr Val Ile Gly Phe Leu Leu Pro Phe Ile Ala Leu
195 200 205

Leu Ala Cys Tyr Cys Arg Met Ala Arg Arg Leu Cys Arg Gln Asp Gly
210 215 220

Pro Ala Gly Pro Val Ala Gln Glu Arg Arg Ser Lys Ala Ala Arg Met
225 230 235 240

Ala Val Val Val Ala Ala Val Phe Ala Ile Ser Phe Leu Pro Phe His
245 250 255

Ile Thr Lys Thr Ala Tyr Leu Ala Val Arg Ser Thr Pro Gly Val Ser
260 265 270

Cys Pro Val Leu Glu Thr Phe Ala Ala Ala Tyr Lys Gly Thr Arg Pro
275 280 285

Phe Ala Ser Val Asn Ser Val Leu Asp Pro Ile Leu Phe Tyr Phe Thr
290 295 300

Gln Gln Lys Phe Arg Arg Gln Pro His Asp Leu Leu Gln Arg Leu Thr
305 310 315 320

Ala Lys Trp Gln Arg Gln Arg Val
325

(2) INFORMATION FOR SEQ ID NO: 40:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 345
 - (B) TYPE: Nucleic acid
 - (C) STRANDEDNESS: Double
 - (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE

(C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

GCTTCCTGGC	ACAAGCGTGG	AGGTCGCCGT	GCTGCTTGGG	TAGTGTGTGG	AGTCGTGTGG	60
CTGGCTGTGA	CAGCCCAGTG	CCTGCCCACG	GCAGTCTTTG	CTGCCACAGG	CATCCAGCGC	120
AACCGCACTG	TGTGCTACGA	CCTGAGCCCA	CCCATCCTGT	CTACTCGCTA	CCTGCCCTAT	180
GGTATGGCCC	TCACGGTCAT	CGGCTTCTTG	CTGCCCTTCA	TAGCCTTACT	GGCTTGTTAT	240
TGTCGCATGG	CCCGCCGCCT	GTGTCGCCAG	GATGGCCCAG	CAGGTCCTGT	GGCCCAAGAG	300
CGGCGCAGCA	AGGCGGCTCG	TATGGCTGTG	GTGGTGGCAG	CTGTC		345

(2) INFORMATION FOR SEQ ID NO: 41:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 984
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Double
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE

(C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

ATGGAGCAGG	ACAATGGCAC	CATCCAGGCT	CCAGGCTTGC	CGCCCACCAC	CTGCGTCTAC	60
CGTGAGGATT	TCAAGCGACT	GCTGCTAACC	CCGGTATACT	CGGTGGTGCT	GGTGGTCGGC	120
CTGCCACTGA	ACATCTGCGT	CATTGCCCAG	ATCTGCGCAT	CCCGCCGGAC	CCTGACCCGT	180
TCCGCTGTGT	ACACCCTGAA	CCTGGCACTG	GCGGACCTGA	TGTATGCCTG	TTCACTACCC	240
CTACTTATCT	ATAACTACGC	CAGAGGGGAC	CACTGGCCCT	TCGGAGACCT	CGCCTGCCGC	300
TTTGTACGCT	TCCTCTTCTA	TGCCAATCTA	CATGGCAGCA	TCCTGTTCTT	CACCTGCATT	360
AGCTTCCAGC	GCTACCTGGG	CATCTGCCAC	CCCCTGGCTT	CCTGGCACAA	GCGTGGAGGT	420
CGCCGTGCTG	CTTGGGTAGT	GTGTGGAGTC	GTGTGGCTGG	CTGTGACAGC	CCAGTGCCTG	480
CCCACGGCAG	TCTTTGCTGC	CACAGGCATC	CAGCGCAACC	GCACTGTGTG	CTACGACCTG	540
AGCCCACCCA	TCCTGTCTAC	TCGCTACCTG	CCCTATGGTA	TGGCCCTCAC	GGTCATCGGC	600
TTCTTGCTGC	CCTTCATAGC	CTTACTGGCT	TGTTATTGTC	GCATGGCCCG	CCGCCTGTGT	660

CGCCAGGATG GCCCAGCAGG TCCTGTGGCC CAAGAGCGGC GCAGCAAGGC GGCTCGTATG 720
 GCTGTGGTGG TGGCAGCTGT CTTTGCCATC AGCTTCCTGC CTTTCCACAT CACCAAGACA 780
 GCCTACTTGG CTGTGCGCTC CACGCCCCGGT GTCTCTTGCC CTGTGCTGGA GACCTTCGCT 840
 GCTGCCTACA AAGGCACTCG GCCCTTCGCC AGTGTCAACA GTGTTCTGGA CCCCATTTCTC 900
 TTCTACTTCA CACAACAGAA GTTCCGGCGG CAACCCCACG ATCTCTTACA GAGGCTCACA 960
 GCCAAGTGGC AGAGGCAGAG AGTC 984

(2) INFORMATION FOR SEQ ID NO: 42:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 128
 (B) TYPE: Amino acid
 (C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 42:

Ala Ala Met Ser Val Asp Arg Tyr Val Ala Ile Val His Ser Arg Arg
 1 5 10 15
 Ser Ser Ser Leu Arg Val Ser Arg Asn Ala Leu Leu Gly Val Gly Phe
 20 25 30
 Ile Trp Ala Leu Ser Ile Ala Met Ala Ser Pro Val Ala Tyr His Gln
 35 40 45
 Arg Leu Phe His Arg Asp Ser Asn Gln Thr Phe Cys Trp Glu Gln Trp
 50 55 60
 Pro Asn Lys Leu His Lys Lys Ala Tyr Val Val Cys Thr Phe Val Phe
 65 70 75 80
 Gly Tyr Leu Leu Pro Leu Leu Leu Ile Cys Phe Cys Tyr Ala Lys Val
 85 90 95
 Leu Asn His Leu His Lys Lys Leu Lys Asn Met Ser Lys Lys Ser Glu
 100 105 110
 Ala Ser Lys Lys Lys Thr Ala Gln Thr Val Leu Val Val Val Val Val
 115 120 125

(2) INFORMATION FOR SEQ ID NO: 43:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 384
 (B) TYPE: Nucleic acid
 (C) STRANDEDNESS: Double
 (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE

(C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 43:

GCCGCGATGT CTGTGGATCG CTACGTGGCC ATTGTGCACT CGCGGCGCTC CTCCTCCCTC 60
AGGGTGTCCC GCAACGCACT GCTGGGCGTG GGCTTCATCT GGGCGCTGTC CATCGCCATG 120
GCCTCGCCGG TGGCCTACCA CCAGCGTCTT TTCCATCGGG ACAGCAACCA GACCTTCTGC 180
TGGGAGCAGT GGCCCAACAA GCTCCACAAG AAGGCTTACG TGGTGTGCAC TTTCGTCTTT 240
GGGTACCTTC TGCCCTTACT GCTCATCTGC TTTTGCTATG CCAAGGTCCT TAATCATCTG 300
CATAAAAAGC TGAAAAACAT GTCAAAAAG TCTGAAGCAT CCAAGAAAAA GACTGCACAG 360
ACCGTCCTGG TGGTCGTTGT AGTA 384

(2) INFORMATION FOR SEQ ID NO: 44:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 71
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

Val Leu Trp Phe Phe Gly Phe Ser Ile Lys Arg Thr Pro Phe Ser Val
1 5 10 15
Tyr Phe Leu His Leu Ala Ser Ala Asp Gly Ala Tyr Leu Phe Ser Lys
20 25 30
Ala Val Phe Ser Leu Leu Asn Ala Gly Gly Phe Leu Gly Thr Phe Ala
35 40 45
His Tyr Val Arg Ser Val Ala Arg Val Leu Gly Leu Cys Ala Phe Val
50 55 60
Ala Gly Val Ser Leu Leu Pro
65 70

(2) INFORMATION FOR SEQ ID NO: 45:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 215
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Double
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE

(C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 45:

GTGCTCTGGT TCTTCGGCTT CTCCATCAAG AGGACCCCCT TCTCCGTCTA CTTCTGCAC 60
 CTGGCCAGCG CCGACGGCGC CTACCTCTTC AGCAAGGCCG TGTTCCTCCT GCTGAACGCC 120
 GGCGGCTTCC TGGGCACCTT CGCCCACTAT GTGCGCAGCG TGGCCCGGGT GCTGGGGCTC 180
 TGCGCCTTCG TGGCGGGCGT GAGCCTCCTG CCGGC 215

(2) INFORMATION FOR SEQ ID NO: 46:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 348
 (B) TYPE: Amino acid
 (C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 46:

Met Glu Leu Ala Met Val Asn Leu Ser Glu Gly Asn Gly Ser Asp Pro
 1 5 10 15
 Glu Pro Pro Ala Pro Glu Ser Arg Pro Leu Phe Gly Ile Gly Val Glu
 20 25 30
 Asn Phe Ile Thr Leu Val Val Phe Gly Leu Ile Phe Ala Met Gly Val
 35 40 45
 Leu Gly Asn Ser Leu Val Ile Thr Val Leu Ala Arg Ser Lys Pro Gly
 50 55 60
 Lys Pro Arg Ser Thr Thr Asn Leu Phe Ile Leu Asn Leu Ser Ile Ala
 65 70 75 80
 Asp Leu Ala Tyr Leu Leu Phe Cys Ile Pro Phe Gln Ala Thr Val Tyr
 85 90 95
 Ala Leu Pro Thr Trp Val Leu Gly Ala Phe Ile Cys Lys Phe Ile His
 100 105 110
 Tyr Phe Phe Thr Val Ser Met Leu Val Ser Ile Phe Thr Leu Ala Ala
 115 120 125
 Met ser Val Asp Arg Tyr Val Ala Ile Val His Ser Arg Arg Ser Ser
 130 135 140
 Ser Leu Arg Val Ser Arg Asn Ala Leu Leu Gly Val Gly Phe Ile Trp
 145 150 155 160
 Ala Leu Ser Ile Ala Met Ala Ser Pro Val Ala Tyr His Gln Arg Leu
 165 170 175

Phe	His	Arg	Asp	Ser	Asn	Gln	Thr	Phe	Cys	Trp	Glu	Gln	Trp	Pro	Asn
			180					185					190		
Lys	Leu	His	Lys	Lys	Ala	Tyr	Val	Val	Cys	Thr	Phe	Val	Phe	Gly	Tyr
		195					200					205			
Leu	Leu	Pro	Leu	Leu	Leu	Ile	Cys	Phe	Cys	Tyr	Ala	Lys	Val	Leu	Asn
	210					215					220				
His	Leu	His	Lys	Lys	Leu	Lys	Asn	Met	Ser	Lys	Lys	Ser	Glu	Ala	Ser
225					230					235					240
Lys	Lys	Lys	Thr	Ala	Gln	Thr	Val	Leu	Val	Val	Val	Val	Val	Phe	Gly
				245					250					255	
Ile	Ser	Trp	Leu	Pro	His	His	Val	Val	His	Leu	Trp	Ala	Glu	Phe	Gly
			260					265					270		
Ala	Phe	Pro	Leu	Thr	Pro	Ala	Ser	Phe	Phe	Phe	Arg	Ile	Thr	Ala	His
		275					280					285			
Cys	Leu	Ala	Tyr	Ser	Asn	Ser	Ser	Val	Asn	Pro	Ile	Ile	Tyr	Ala	Phe
	290					295					300				
Leu	Ser	Glu	Asn	Phe	Arg	Lys	Ala	Tyr	Lys	Gln	Val	Phe	Lys	Cys	His
305					310					315					320
Val	Cys	Asp	Glu	Ser	Pro	Arg	Ser	Glu	Thr	Lys	Glu	Asn	Lys	Ser	Arg
				325				330						335	
Met	Asp	Thr	Pro	Pro	Ser	Thr	Asn	Cys	Thr	His	Val				
			340					345							

(2) INFORMATION FOR SEQ ID NO: 47:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1044
 - (B) TYPE: Nucleic acid
 - (C) STRANDEDNESS: Double
 - (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE

- (C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 47:

ATGGA	ACTGG	CTATG	GTGAA	CCTCA	GTGAA	GGAAT	GGA	GCGAC	CCAGA	GCCGC	CAGCC		60
CCGGAG	TCCA	GGCCG	CTCTT	CGGCAT	TGGC	GTGGAG	AACT	TCATT	ACGCT	GGTAG	TGTTT		120
GGCCT	GATTT	TCGCG	ATGGG	CGTGCT	GGGC	AACAG	CCTGG	TGATC	ACCGT	GCTGG	CGCGC		180
AGCAA	ACCAG	GCAAC	CCCCG	CAGCAC	CACC	AACCT	GTTTA	TCCTC	AATCT	GAGCA	TCGCA		240

GACCTGGCCT ACCTGCTCTT CTGCATCCCT TTTCAGGCCA CCGTGTATGC ACTGCCCACC 300
TGGGTGCTGG GCGCCTTCAT CTGCAAGTTT ATACACTACT TCTTCACCGT GTCCATGCTG 360
GTGAGCATCT TCACCCTGGC CGCGATGTCT GTGGATCGCT ACGTGGCCAT TGTGCACTCG 420
CGGCGCTCCT CCTCCCTCAG GGTGTCCCGC AACGCACTGC TGGGCGTGGG CTTTCATCTGG 480
GCGCTGTCCA TCGCCATGGC CTCGCCGGTG GCCTACCACC AGCGTCTTTT CCATCGGGAC 540
AGCAACCAGA CCTTCTGCTG GGAGCAGTGG CCCAACAAGC TCCACAAGAA GGCTTACGTG 600
GTGTGCACTT TCGTCTTTGG GTACCTTCTG CCCTTACTGC TCATCTGCTT TTGCTATGCC 660
AAGGTCCTTA ATCATCTGCA TAAAAAGCTG AAAAACATGT CAAAAAAGTC TGAAGCATCC 720
AAGAAAAAGA CTGCACAGAC CGTCCTGGTG GTCGTTGTAG TATTTGGCAT ATCCTGGCTG 780
CCCCATCATG TCGTCCACCT CTGGGCTGAG TTTGGAGCCT TCCCACTGAC GCCAGCTTCC 840
TTCTTCTTCA GAATCACCGC CCATTGCCTG GCATACAGCA ACTCCTCAGT GAACCCCATC 900
ATATATGCCT TTCTCTCAGA AACTTCCGG AAGGCGTACA AGCAAGTGTT CAAGTGTCAT 960
GTTTGCGATG AATCTCCACG CAGTGAAACT AAGGAAAACA AGAGCCGGAT GGACACCCCG 1020
CCATCCACCA ACTGCACCCA CGTG 1044

(2) INFORMATION FOR SEQ ID NO: 48:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 125
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 48:

Leu Leu Thr Leu His Pro Val Trp Ser Gln Lys His Arg Thr Ser His
1 5 10 15
Trp Ala Ser Arg Val Val Leu Gly Val Trp Leu Ser Ala Thr Ala Phe
20 25 30
Ser Val Pro Tyr Leu Val Phe Arg Glu Thr Tyr Asp Asp Arg Lys Gly
35 40 45
Arg Val Thr Cys Arg Asn Asn Tyr Ala Val Ser Thr Asp Trp Glu Ser
50 55 60
Lys Glu Met Gln Thr Val Arg Gln Trp Ile His Ala Thr Cys Phe Ile
65 70 75 80
Ser Arg Phe Ile Leu Gly Phe Leu Leu Pro Phe Leu Val Ile Gly Phe

				85					90					95					
Cys	Tyr	Glu	Arg	Val	Ala	Arg	Lys	Met	Lys	Glu	Arg	Gly	Leu	Phe	Lys				
				100				105					110						
Ser	Ser	Lys	Pro	Phe	Lys	Val	Thr	Met	Thr	Ala	Val	Ile							
		115					120					125							

(2) INFORMATION FOR SEQ ID NO: 49:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 377
 - (B) TYPE: Nucleic acid
 - (C) STRANDEDNESS: Double
 - (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE

(C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 49:

CTTCTCACCC TTCACCCAGT GTGGTCCCAA AAGCACCGAA CCTCACACTG GGCTTCCAGA	60
GTCGTTCTGG GAGTCTGGCT CTCTGCCACT GCCTTCAGCG TGCCCTATTT GGTTTTCAGG	120
GAGACATATG ATGACCGTAA AGGAAGAGTG ACCTGCAGAA ATAACTACGC TGTGTCCACT	180
GACTGGGAAA GCAAAGAGAT GCAAACAGTA AGACAATGGA TTCATGCCAC CTGTTTCATC	240
AGCCGCTTCA TACTGGGCTT CCTTCTGCCT TTCTTAGTCA TTGGCTTTTG TTATGAAAGA	300
GTAGCCCGCA AGATGAAAGA GAGGGGCCTC TTAAATCCA GCAAACCCTT CAAAGTCACG	360
ATGACTGCTG TTATCTC	377

(2) INFORMATION FOR SEQ ID NO: 50:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 119
 - (B) TYPE: Amino acid
 - (C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 50:

Phe	Lys	Ile	Val	Lys	Pro	Leu	Ser	Thr	Ser	Phe	Ile	Gln	Ser	Val	Asn
1				5					10					15	
Tyr	Ser	Lys	Leu	Val	Ser	Leu	Val	Val	Trp	Leu	Leu	Met	Leu	Leu	Leu
			20					25					30		
Ala	Val	Pro	Asn	Val	Ile	Leu	Thr	Asn	Gln	Arg	Val	Lys	Asp	Val	Thr
		35					40					45			

Gln Ile Lys Cys Met Glu Leu Lys Asn Glu Leu Gly Arg Gln Trp His
 50 55 60
 Lys Ala Ser Asn Tyr Ile Phe Val Gly Ile Phe Trp Leu Val Phe Leu
 65 70 75 80
 Leu Leu Ile Ile Phe Tyr Thr Ala Ile Thr Arg Lys Ile Phe Lys Ser
 85 90 95
 His Leu Lys Ser Arg Lys Asn Ser Ile Ser Val Lys Lys Lys Ser Ser
 100 105 110
 Arg Asn Ile Phe Ser Ile Val
 115

(2) INFORMATION FOR SEQ ID NO: 51:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 357
 (B) TYPE: Nucleic acid
 (C) STRANDEDNESS: Double
 (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cdna

(ix) FEATURE
 (C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 51:

TTCAAGATTG TGAAGCCCCT TTCCACGTCC TTCATCCAGT CTGTGAACTA CAGCAAACCTC 60
 GTCTCGCTGG TGGTCTGGTT GCTCATGCTC CTCCTCGCCG TCCCCAACGT CATTCTCACC 120
 AACCAGAGAG TTAAGGACGT GACGCAGATA AAATGCATGG AACTTAAAAA CGAACTGGGC 180
 CGCCAGTGGC ACAAGGCGTC AACTACATC TTTGTGGGCA TTTTCTGGCT TGTGTTTCCTT 240
 TTGCTAATCA TTTTCTACAC TGCTATCACC AGGAAAATCT TTAAGTCCCA CCTGAAATCC 300
 AGAAAGAATT CCATCTCGGT CAAAAGAAA TCTAGCCGCA ACATCTTCAG CATCGTG 357

(2) INFORMATION FOR SEQ ID NO: 52:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 252
 (B) TYPE: Amino acid
 (C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 52:

Val Asp Leu Leu Ala Ala Leu Thr Leu Met Pro Leu Ala Met Leu Ser
 1 5 10 15

Ser Ser Ala Leu Phe Asp His Ala Leu Phe Gly Glu Val Ala Cys Arg
20 25 30

Leu Tyr Leu Phe Leu Ser Val Cys Phe Val Ser Leu Ala Ile Leu Ser
35 40 45

Val Ser Ala Ile Asn Val Glu Arg Tyr Tyr Tyr Val Val His Pro Met
50 55 60

Arg Tyr Glu Val Arg Met Lys Leu Gly Leu Val Ala Ser Val Leu Val
65 70 75 80

Gly Val Trp Val Lys Ala Leu Ala Met Ala Ser Val Pro Val Leu Gly
85 90 95

Arg Val Ser Trp Glu Glu Gly Pro Pro Ser Val Pro Pro Gly Cys Ser
100 105 110

Leu Gln Trp Ser His Ser Ala Tyr Cys Gln Leu Phe Val Val Val Phe
115 120 125

Ala Val Leu Tyr Phe Leu Leu Pro Leu Leu Leu Ile Leu Val Val Tyr
130 135 140

Cys Ser Met Phe Arg Val Ala Arg Val Ala Ala Met Gln His Gly Pro
145 150 155 160

Leu Pro Thr Trp Met Glu Thr Pro Arg Gln Arg Ser Glu Ser Leu Ser
165 170 175

Ser Arg Ser Thr Met Val Thr Ser Ser Gly Ala Pro Gln Thr Thr Pro
180 185 190

His Arg Thr Phe Gly Gly Gly Lys Ala Ala Val Val Leu Leu Ala Val
195 200 205

Gly Gly Gln Phe Leu Leu Cys Trp Leu Pro Tyr Phe Ser Phe His Leu
210 215 220

Tyr Val Ala Leu Ser Ala Gln Pro Ile Ala Ala Gly Gln Val Glu Asn
225 230 235 240

Val Val Thr Trp Ile Gly Tyr Phe Cys Phe Thr Ser
245 250

(2) INFORMATION FOR SEQ ID NO: 53:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 756
 - (B) TYPE: Nucleic acid
 - (C) STRANDEDNESS: Double
 - (D) TOPOLOGY: Linear

- (ii) MOLECULE TYPE: cDNA

(ix) FEATURE

(C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 53:

GTGGACCTGC TGGCTGCCCT GACCCTCATG CCTCTGGCCA TGCTCTCCAG CTCCGCCCTC 60
TTTGACCACG CCCTCTTTGG GGAGGTGGCC TGCCGCCTCT ACTTGTTCTT GAGCGTCTGC 120
TTTGTTCAGCC TGGCCATCCT CTCGGTGTCC GCCATCAATG TGGAGCGCTA CTATTATGTG 180
GTCCACCCCA TGCCTATGA GGTGCGCATG AACTGGGGC TGGTGGCCTC TGTGCTGGTG 240
GGCGTGTGGG TGAAGGCCCT GGCCATGGCT TCTGTGCCAG TGTGGAAG GGTGTCCTGG 300
GAGGAAGGCC CTCCCAGTGT CCCCCAGGC TGTTCACTCC AATGGAGCCA CAGTGCCTAC 360
TGCCAGCTTT TCGTGGTGGT CTTGCGCGTC CTCTACTTCC TGCTGCCCCT GCTCCTCATC 420
CTTGTGGTCT ACTGCAGCAT GTTCCGGGTG GCTCGTGTGG CTGCCATGCA GCACGGGCCG 480
CTGCCCACGT GGATGGAGAC GCCCCGGCAA CGCTCCGAGT CTCTCAGCAG CCGCTCCACT 540
ATGGTCACCA GCTCGGGGGC CCCGCAGACC ACCCCTCACC GGACGTTTGG CGGAGGGAAG 600
GCAGCAGTGG TCCTCCTGGC TGTGGGAGGA CAGTTCCTGC TCTGTTGGTT GCCCTACTTC 660
TCCTTCCACC TCTATGTGGC CCTGAGCGCT CAGCCATTG CAGCGGGGCA GGTGGAGAAC 720
GTGGTGACCT GGATTGGCTA CTTCTGCTTC ACCTCC 756

(2) INFORMATION FOR SEQ ID NO: 54:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 263
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 54:

Ala Asp Val Leu Val Thr Ala Ile Cys Leu Pro Ala Ser Leu Leu Val
1 5 10 15
Asp Ile Thr Glu Ser Trp Leu Phe Gly His Ala Leu Cys Lys Val Ile
20 25 30
Pro Tyr Leu Gln Ala Val Ser Val Ser Val Val Val Leu Thr Leu Ser
35 40 45
Ser Ile Ala Leu Asp Arg Trp Tyr Ala Ile Cys His Pro Leu Leu Phe
50 55 60
Lys Ser Thr Ala Arg Arg Ala Arg Gly Ser Ile Leu Gly Ile Trp Ala
65 70 75 80

Val Ser Leu Ala Val Met Val Pro Gln Ala Ala Val Met Glu Cys Ser
85 90 95

Ser Val Leu Pro Glu Leu Ala Asn Arg Thr Arg Leu Leu Ser Val Cys
100 105 110

Asp Glu Arg Trp Ala Asp Asp Leu Tyr Pro Lys Ile Tyr His Ser Cys
115 120 125

Phe Phe Ile Val Thr Tyr Leu Ala Pro Leu Gly Leu Met Ala Met Ala
130 135 140

Tyr Phe Gln Ile Phe Arg Lys Leu Trp Gly Arg Gln Ile Pro Gly Thr
145 150 155 160

Thr Ser Ala Leu Val Arg Asn Trp Lys Arg Pro Ser Asp Gln Leu Asp
165 170 175

Asp Gln Gly Gln Gly Leu Ser Ser Glu Pro Gln Pro Arg Ala Arg Ala
180 185 190

Phe Leu Ala Glu Val Lys Gln Met Arg Ala Arg Arg Lys Thr Ala Lys
195 200 205

Met Leu Met Val Val Leu Leu Val Phe Ala Leu Cys Tyr Leu Pro Ile
210 215 220

Ser Val Leu Asn Val Leu Lys Arg Val Phe Gly Met Phe Arg Gln Ala
225 230 235 240

Ser Asp Arg Glu Ala Ile Tyr Ala Cys Phe Thr Phe Ser His Trp Leu
245 250 255

Val Tyr Ala Asn Ser Ala Ala
260

(2) INFORMATION FOR SEQ ID NO: 55:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 789
 (B) TYPE: Nucleic acid
 (C) STRANDEDNESS: Double
 (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE
 (C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 55:

GCCGATGTGC TGGTGACAGC CATCTGCCTG CCGGCCAGTC TGCTGGTAGA CATCACGGAA 60
 TCCTGGCTCT TTGGCCATGC CCTCTGCAAG GTCATCCCCT ATCTACAGGC CGTGTCCGTG 120

TCAGTGGTCG TGCTGACTCT CAGCTCCATC GCCCTGGACC GCTGGTACGC CATCTGCCAC 180
 CCGCTGTTGT TCAAGAGCAC TGCCCGGCGC GCCCGCGGCT CCATCCTCGG CATCTGGGCG 240
 GTGTCGCTGG CTGTCATGGT GCCTCAGGCT GCTGTCATGG AGTGTAGCAG CGTGCTGCCC 300
 GAGCTGGCCA ACCGCACCCG CCTCCTGTCT GTCTGTGATG AGCGCTGGGC AGACGACCTG 360
 TACCCCAAGA TCTACCACAG CTGCTTCTTC ATTGTCACCT ACCTGGCCCC ACTGGGCCTC 420
 ATGGCCATGG CCTATTTCCA GATCTTCCGC AAGCTCTGGG GCCGCCAGAT CCCCGGCACC 480
 ACCTCGGCCC TGGTGCGCAA CTGGAAGCGG CCCTCAGACC AGCTGGACGA CCAGGGCCAG 540
 GGCCTGAGCT CAGAGCCCCA GCCCCGGGCC CGCGCCTTCC TGGCCGAGGT GAAACAGATG 600
 CGAGCCCCGA GGAAGACGGC CAAGATGCTG ATGGTGGTGC TGCTGGTCTT CGCCCTCTGC 660
 TACCTGCCCCA TCAGTGTCTT CAACGTCCTC AAGAGGGTCT TCGGGATGTT CCGCCAAGCC 720
 AGCGACCGAG AGGCCATCTA CGCCTGCTTC ACCTTCTCCC ACTGGCTGGT GTACGCCAAC 780
 AGCGCCGCC 789

(2) INFORMATION FOR SEQ ID NO: 56:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 328
 (B) TYPE: Amino acid
 (C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 56:

Met Glu Trp Asp Asn Gly Thr Gly Gln Ala Leu Gly Leu Pro Pro Thr
 1 5 10 15
 Thr Cys Val Tyr Arg Glu Asn Phe Lys Gln Leu Leu Leu Pro Pro Val
 20 25 30
 Tyr Ser Ala Val Leu Ala Ala Gly Leu Pro Leu Asn Ile Cys Val Ile
 35 40 45
 Thr Gln Ile Cys Thr Ser Arg Arg Ala Leu Thr Arg Thr Ala Val Tyr
 50 55 60
 Thr Leu Asn Leu Ala Leu Ala Asp Leu Leu Tyr Ala Cys Ser Leu Pro
 65 70 75 80
 Leu Leu Ile Tyr Asn Tyr Ala Gln Gly Asp His Trp Pro Phe Gly Asp
 85 90 95
 Phe Ala Cys Arg Leu Val Arg Phe Leu Phe Tyr Ala Asn Leu His Gly
 100 105 110

Ser Ile Leu Phe Leu Thr Cys Ile Ser Phe Gln Arg Tyr Leu Gly Ile
115 120 125

Cys His Pro Leu Ala Pro Trp His Lys Arg Gly Gly Arg Arg Ala Ala
130 135 140

Trp Leu Val Cys Val Thr Val Trp Leu Ala Val Thr Thr Gln Cys Leu
145 150 155 160

Pro Thr Ala Ile Phe Ala Ala Thr Gly Ile Gln Arg Asn Arg Thr Val
165 170 175

Cys Tyr Asp Leu Ser Pro Pro Ala Leu Ala Thr His Tyr Met Pro Tyr
180 185 190

Gly Met Ala Leu Thr Val Ile Gly Phe Leu Leu Pro Phe Ala Ala Leu
195 200 205

Leu Ala Cys Tyr Cys Leu Leu Ala Cys Arg Leu Cys Arg Gln Asp Gly
210 215 220

Pro Ala Glu Pro Val Ala Gln Glu Arg Arg Gly Lys Ala Ala Arg Met
225 230 235 240

Ala Val Val Val Ala Ala Ala Phe Ala Ile Ser Phe Leu Pro Phe His
245 250 255

Ile Thr Lys Thr Ala Tyr Leu Ala Val Gly Ser Thr Pro Gly Val Pro
260 265 270

Cys Thr Val Leu Glu Ala Phe Ala Ala Ala Tyr Lys Gly Thr Arg Pro
275 280 285

Phe Ala Ser Ala Asn Ser Val Leu Asp Rro Ile Leu Phe Tyr Phe Thr
290 295 300

Gln Lys Lys Phe Arg Arg Arg Pro His Glu Leu Leu Gln Lys Leu Thr
305 310 315 320

Ala Lys Trp Gln Arg Gln Gly Arg
325

(2) INFORMATION FOR SEQ ID NO: 57:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 984
- (B) TYPE: Nucleic acid
- (C) STRANDEDNESS: Double
- (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE

- (C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 57:

ATGGAATGGG ACAATGGCAC AGGCCAGGCT CTGGGCTTGC CACCCACCAC CTGTGTCTAC	60
CGCGAGAACT TCAAGCAACT GCTGCTGCCA CCTGTGTATT CGGCGGTGCT GGCGGCTGGC	120
CTGCCGCTGA ACATCTGTGT CATTACCCAG ATCTGCACGT CCCGCCGGGC CCTGACCCGC	180
ACGGCCGTGT ACACCCTAAA CCTTGCTCTG GCTGACCTGC TATATGCCTG CTCCCTGCCC	240
CTGCTCATCT ACAACTATGC CCAAGGTGAT CACTGGCCCT TTGGCGACTT CGCCTGCCGC	300
CTGGTCCGCT TCCTCTTCTA TGCCAACCTG CACGGCAGCA TCCTCTTCCT CACCTGCATC	360
AGCTTCCAGC GCTACCTGGG CATCTGCCAC CCGCTGGCCC CCTGGCACAA ACGTGGGGGC	420
CGCCGGGCTG CCTGGCTAGT GTGTGTAACC GTGTGGCTGG CCGTGACAAC CCAGTGCCTG	480
CCCACAGCCA TCTTCGCTGC CACAGGCATC CAGCGTAACC GCACTGTCTG CTATGACCTC	540
AGCCCGCCTG CCCTGGCCAC CCACTATATG CCCTATGGCA TGGCTCTCAC TGTCATCGGC	600
TTCCTGCTGC CCTTTGCTGC CCTGCTGGCC TGCTACTGTC TCCTGGCCTG CCGCCTGTGC	660
CGCCAGGATG GCCCGGCAGA GCCTGTGGCC CAGGAGCGGC GTGGCAAGGC GGCCCGCATG	720
GCCGTGGTGG TGGCTGCTGC CTTTGCCATC AGCTTCCTGC CTTTTACAT CACCAAGACA	780
GCCTACCTGG CAGTGGGCTC GACGCCGGGC GTCCCCTGCA CTGTATTGGA GGCCTTTGCA	840
GCGGCCTACA AAGGCACGCG GCCGTTTGCC AGTGCCAACA GCGTGCTGGA CCCCATCCTC	900
TTCTACTTCA CCCAGAAGAA GTTCCGCCGG CGACCACATG AGCTCCTACA GAAACTCACA	960
GCCAAATGGC AGAGGCAGGG TCGC	984

(2) INFORMATION FOR SEQ ID NO: 58:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26
- (B) TYPE: Nucleic acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

- (ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 58:

ACAGCCATCT TCGCTGCCAC AGGCAT 26

(2) INFORMATION FOR SEQ ID NO: 59:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29
- (B) TYPE: Nucleic acid
- (C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 59:

AGACAGTAGC AGGCCAGCAG GGCAGCAAA 29

(2) INFORMATION FOR SEQ ID NO: 60:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(iii) FEATURES: N is inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 60:

CTGTGYGYSA TYGCNNTKGA YMGSTAC 27

(2) INFORMATION FOR SEQ ID NO: 61:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(iii) FEATURES: N is inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 61:

AKGWAGWAGG GCAGCCAGCA GANSRYGAA 29

CLAIMS

1. A DNA which comprises a nucleotide sequence represented by a SEQ ID NO selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 19.

5 2. A method for amplifying a DNA coding for a G protein coupled receptor protein by polymerase chain reaction techniques, which comprises:

(i) carrying out a polymerase chain reaction in the presence of a mixture of

- 10 ① a DNA coding for a G protein coupled receptor protein, said DNA being capable of acting as a template,
- 15 ② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 1, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 3, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 5, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 6, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 7, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 10, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 14, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 16 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 18, and
- 20 ③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 2, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 4, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 8, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 9, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 11, DNA primers comprising a nucleotide
- 25
- 30

sequence represented by SEQ ID NO: 15, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 19; or

5 (ii) carrying out a polymerase chain reaction in the presence of a mixture of

- ① a DNA coding for G protein coupled receptor protein, said DNA being capable of acting as a template,
- ② at least one DNA primer selected from the group
10 consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 1 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 12, and
- ③ at least one DNA primer selected from the group
15 consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 13.

3. A method for screening a DNA library for a DNA coding for a G protein coupled receptor protein, which comprises:

20 (i) carrying out a polymerase chain reaction in the presence of a mixture of

- ① said DNA library,
- ② at least one DNA primer selected from the group
25 consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 1, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 3, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 5, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 6, DNA
30 primers comprising a nucleotide sequence represented by SEQ ID NO: 7, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 10, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 14, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 16 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 18, and
35
- ③ at least one DNA primer selected from the group

consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 2, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 4, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 8, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 9, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 11, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 15, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 19,

under conditions to amplify selectively a template DNA coding for the G protein coupled receptor protein, contained in the DNA library and selecting said DNA; or

(ii) carrying out a polymerase chain reaction in the presence of a mixture of

① said DNA library

② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 1 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 12, and

③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 13,

under conditions to amplify selectively a DNA coding for the G protein coupled receptor protein, contained in the DNA library and selecting said DNA.

4. A DNA coding for a G protein coupled receptor protein or a fragment thereof, which is obtained by the method according to claim 2 to 3.

5. A G protein coupled receptor protein encoded by the DNA according to claim 4, a peptide segment or fragment thereof or a salt thereof.

6. A G protein coupled receptor protein comprising an amino acid sequence selected from the group consisting of

an amino acid sequence represented by SEQ ID NO: 24, an amino acid sequence represented by SEQ ID NO: 25, an amino acid sequence represented by SEQ ID NO: 26, an amino acid sequence represented by SEQ ID NO: 27, an amino acid sequence represented by SEQ ID NO: 28, an amino acid sequence represented by SEQ ID NO: 34, an amino acid sequence represented by SEQ ID NO: 35, an amino acid sequence represented by SEQ ID NO: 38, an amino acid sequence represented by SEQ ID NO: 39, an amino acid sequence represented by SEQ ID NO: 56, and substantial equivalents to the amino acid sequence represented by SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 38, SEQ ID NO: 39, or SEQ ID NO: 56; a peptide segment (or fragment) thereof, a modified peptide derivative thereof or a salt thereof.

7. The G protein coupled receptor protein according to claim 6, comprising an amino acid sequence selected from the group consisting of an amino acid sequence represented by SEQ ID NO: 38, an amino acid sequence represented by SEQ ID NO: 39, an amino acid sequence represented by SEQ ID NO: 56 and substantial equivalents to the amino acid sequence represented by SEQ ID NO: 38, SEQ ID NO: 39, or SEQ ID NO: 56.

8. The G protein coupled receptor protein according to claims 6 or 7, wherein said receptor is a purinoceptor.

9. The G protein coupled receptor protein according to any of claims 6 to 8, wherein an agonist to said receptor is useful as an immunomodulator or an antitumor agent, in addition it is useful in therapeutically or prophylactically treating hypertension, diabetes or cystic fibrosis, and an antagonist to said receptor is useful as a hypotensive agent, an analgesic, or an agent for therapeutically or prophylactically treating incontinence of urine.

10. A DNA which comprises a nucleotide sequence coding for a G protein coupled receptor protein of claim 6.

11. The DNA according to claim 10 comprising a nucleotide sequence coding for the G protein coupled receptor

protein according to claim 7.

12. The DNA according to claim 11 comprising a nucleotide sequence represented by SEQ ID NO: 40, SEQ ID NO: 41, or SEQ ID NO: 57.

5 13. A transformant containing a vector comprising the DNA according to claim 4 or 10; or an expression system comprising an open reading frame (ORF) of DNA derived from a G protein coupled receptor protein DNA according to claim 4 or 10, wherein the ORF is operably linked
10 to a control sequence compatible with a desired host cell.

14. A method for determining a ligand to the G protein coupled receptor protein according to any of claims 5 to 8, which comprises contacting

(i) at least one component selected from the group
15 consisting of G protein coupled receptor proteins or salts thereof according to any of claims 5 to 8, peptide segments or salts thereof, and mixtures thereof,
with

(ii) at least one compound to be tested
20 and determining whether said compound to be tested bound to the component of (i).

15. A screening method for a compound capable of inhibiting the binding of a G protein coupled receptor protein according to any of claims 5 to 8 with a ligand, which
25 comprises carrying out a comparison between:

(i) at least one case where said ligand is contacted with at least one component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of claims 5 to 8,
30 peptide segments or salts thereof, and mixtures thereof,
and

(ii) at least one case where said ligand together with a compound to be tested is contacted with at least one component selected from the group consisting
35 of G protein coupled receptor proteins or salts thereof according to any of claims 5 to 8, peptide segments or salts thereof, and mixtures thereof.

16. A compound which is determined through the method according to claim 15 or a salt thereof.

17. The compound according to claim 16, which is an agonist or antagonist to a G protein coupled receptor protein according to any of claims 5 to 8.

18. A ligand to a G protein coupled receptor protein according to any of claims 5 to 8, which is determined through the method according to claim 14.

10

15

ABSTRACT

DNA primers effective in screening G protein coupled receptor protein-encoding DNA fragments are provided. The primers which are complementary to nucleotide sequences that are in community
5 with (homologous to) the nucleotide sequences encoding amino acid sequences corresponding to or near the first membrane-spanning domain or the sixth membrane-spanning domain each of known various G protein coupled receptor proteins were designed and synthesized. Methods of amplifying G protein coupled
10 receptor protein-encoding DNAs using the above DNA primers, and novel target G protein coupled receptor protein-encoding DNAs are also provided. Screening of DNA libraries can be efficiently carried out. Human pituitary gland or amygdala-derived and mouse pancreas-derived G protein coupled receptor
15 proteins, etc. or salts thereof, partial peptides thereof, DNAs coding for the above G protein coupled receptor proteins, processes for producing the above G protein coupled receptor proteins, methods of determining ligands for the above G protein coupled receptor proteins, methods of screening
20 compounds that inhibit the binding between the ligand and the G protein coupled receptor proteins or screening kits therefor, compounds or salts thereof obtained by the above screening method or the screening kit, pharmaceutical compositions containing the above compounds or salts thereof, and antibodies
25 against the above protein coupled receptor proteins or partial peptides thereof are provided.

FIGURE 1

OLIGODEOXYNUCLEOTIDE SEQUENCE FOR 5' SIDE PRIMER

Primer HS-1	CGTGGCCATCCTGGGCAACACCCTG
	G C GG CT
	G
	T
HTRHR	CCTGGGCATTGTAGGCAACATCATGGT
HUMRANTES	CATTGGCCTGGTTGGAAACATCCTGGT
HSBLR1A	CCTGGGCGTGATCGGCAACGTCCTGGT
HUMSOMAT	GGTGGGGCTGGTGGGCAACGCCCTGGT
RNU02083	AGTGGGCCTCTTCGGAAACTTCCTGGT
U00442	GGTGGGCTTAGTGGGCAATTCCTGGT
HUMNMBR	CGTGGGCTTGCTGGGCAACATCATGCT
HSBM4	GGTGACCATCATCGGCAACATCCTGGT
RATAADRE01	CTTTGCCATCGTGGGCAACATCTTGGT
HUMSSTR3X	GGTGGGCCTGCTGGGTAACCTCGCTGGT
HUMC5AAR	GGTGGGAGTGCTGGGCAATGCCCTGGT
HUMRDC1A	CATCGGCATGATTGCCAACTCCGTGGT
HUMOPIODRE	CGTGGCGGTGCTCGGCAACCTCGTGGT
RATA2BAR	GCTGGCAGTGGCGGGCAACGTGCTGGT

FIGURE 2

OLIGODEOXYNUCLEOTIDE SEQUENCE FOR 3' SIDE PRIMER

Complementary Sequence	TTTGCCATCTGCTGGATGCCCCACAAC
to Primer HS-2	<div> <div>C</div> <div>C</div> <div>TTT C</div> </div> <div> <div>G</div> <div>G</div> </div> <div> <div>T</div> <div>T</div> </div>

HUMSGIR	TTTGCCCTCTGCTGGTTCCCTCTCAAC
HUMBOMB3S	TTTGCCCTCTGCTGGTTGCCAAATCAC
S46950	TTTGCCCTCTGCTGGCTGCCCTACAC
MUSGPCR	TTTGCCCTCGTCTGGTGCCCTCTCAAC
S43387	TTTGCCCTTTTATGGATGCCCTACAGG
RATNEURA	TTTGCCATCTGCTGGCTGCCCTATCAC
RATA1ARA	TTTGCCCTCAGCTGGCTGCCGCTGCAT
HUMOPIODRE	TTTGCCATCTGCTGGCTGCCCTATCAC
HUMNEKAR	TTTGCCATCTGCTGGCTGCCCTACCAC
RATADENREC	TTTGCCCTTGTGCTGGCTGCCTTTGTCC
HUMSRI1A	TTTGTCATCTGCTGGATGCCTTTCTAC
S8637154	TTTGCTATCTGCTGGCTGCCCTATCAT
RNCGPCR	TTTGCCGCCTGCTGGATGCCTTTTACC
HUMSSTR4Z	TTTGTGCTCTGCTGGATGCCTTTCTAC
RATGNRHA	TTTGCACTGCTCGAAGCCAGACAAA

FIGURE 3

OLIGODEOXYNUCLEOTIDE SEQUENCE FOR 5' SIDE PRIMER

Primer 3A CTGACCGCTCTIACIACTGACCGATAC
 T T GG GT A C
 G

Primer 3B CTGACCGCTCTIACIACTGACCGATAT
 T T GG GT A C
 G

L11064	CTCACCATGATGAGCGTGGACCGCTAC
L11065	TTGACCATGATGGAGTGTGACCGCTAC
D16349	CTCTGCACCATGAGCGTGGACCGCTAC
X69676	CTGATGCTCGTGAGTATCGACCGCTAC
M35328	CTTACGGCACTGTCAGCTGACAGGTAC
M73482	CTCACTGCCCTCAGCGCCGACAGGTAC
M73481	CTCACGGCGCTCTCGGCAGACAGATAC
L08893	TTAACAATTCTCAGCGCTGACAGATAC
X62933	ATGACCGCCATCGCCGCTGACAGGTAC
X62934	ATGACAACTGTGGCCTTTGACAGATAC
J05189	ATGACAGCCATTGCAGTGGACAGGTAT
M60786	CTCTGCGCTCTCAGTGTGGACAGGTAC
L04672	CTCACCTGCCTCAGCATTGACCGCTAC
X61496	TTGCTGGCTATCACTGTGGACCGCTAC
X59249	TTGCTGGCCATTGCTGTAGACCGATAC
L09249	CTCACCTGCCTCAGCATTGACCGCTAC
P30731	CTGACAGCTATCGCAGTGGACCGCCAC
M31210	CTCCTCGCCATCGCCATTGAGCGCTAT
U03642	CTCACCGGCCTCAGCTTCGACCGCTAC

FIGURE 4

OLIGODEOXYNUCLEOTIDE SEQUENCE FOR 5' SIDE PRIMER

Primer 3C CTCGCCGCTATIAGCATGGACCGITAC
 G CC G T T

Primer 3D CTCGCCGCTATIAGCATGGACCGITAT
 G CC G T T

L32840 ATTACCTGCATGAGTGTCGATAGGTAC
X64052 CTCACGTGTCTCAGCATCGATCGCTAC
M90065 CTCACGTGTCTCAGCATCGATCGCTAC
M91464 CTCACGTGTCTCAGCATTGATCGATAC
M88096 CTGGTAGCCATCTCTCTGGAGAGATAT
M99418 CTCGTGGCCATAGCCCTGGAGCGATAC
L04473 CTCGTGGCCATCGCACTGGAGCGGTAC
M73969 CTGGCCTGCATCAGTGTGGACCGTTAC
X65858 TTGGCCTGCATCAGTGTGGACCGTTAC
S46665 CTGGCTACCATTAGTGCCGACCGTTTC
M60626 ATCGCCCTCATTGCTCTGGACCGCTGT

FIGURE 5

OLIGODEOXYNUCLEOTIDE SEQUENCE FOR 3' SIDE PRIMER

Complementary Sequence TTTACCITCTGTTGGICGCCCTACCACATC
to Primer 6A GT TC T T

Complementary Sequence TTCACCITCTGTTGGICGCCCTACCACATC
to Primer 6B GT TC T T

L11064	TTCGTGGTGTGCTGGGCGCCCATCCACATC
L11065	TTCATCATCTGTTGGACCCCCATTACATC
D16349	TTTATCGTCTGCTGGACCCCCATCCACATC
X69676	TTTGTGCTGTGTTGGGTGCCTTTCCAGATC
M35328	TTTGCCCTTCTGCTGGCTCCCCAACCATGTC
M73482	TTCATCTTCTGTTGGTTTCCAAACCACATC
M73481	TTCGCCCTTCTGCTGGCTCCCCAATCATGTC
L08893	TTTGCCCTCTGCTGGTTGCCAAATCACCTC
X62933	TTTGCCATCTGCTGGCTGCCCTACCACCTC
X62934	TTCGCCATCTGCTGGCTGCCCTTCCACATC
J05189	TTTGCCATCTGCTGGCTGCCCTATCACGTG
M60786	TTCGCCCTGTGCTGGTTCCCTCTTCACTTA
L04672	TTTGTCATCTGCTGGCTGCCCTACCACGTG
X61496	TTTGCCGCCTGCTGGATGCCCTTTTACCCTC
X59249	TTTGCCCTTGTGCTGGCTGCCCTTTGTCCATC
L09249	TTTGCCATCTGCTGGCTGCCCTACCACGTG
P30731	TTTGCCCTCTGCTGGTTCCCTCTCAACTGC
M31210	TTCATCGCCTGCTGGGCACCGCTCTTCATC
U03642	TTTGCCCTGTGCTGGATGCCCTACCACCTG

FIGURE 6

OLIGODEOXYNUCLEOTIDE SEQUENCE FOR 3' SIDE PRIMER

Complementary Sequence TTTTTCITTTGCTGGITTCCTACCATG
to Primer 6C CC T G C T T

L32840	TTCATCATTTGCTGGCTTCCCTTCCATGTT
X64052	TTCTTCTTTTCCTGGGTTCCCCACCAAATA
M90065	TTCTTCTTTTCCTGGGTTCCCCACCAAATA
M91464	TTTTTCTTTTCCTGGATTCCCCACCAAATA
M88096	TTCTTCCTGTGCTGGATGCCCATCTTCAGC
M99418	TTCTTCCTGTGTTGGCTGCCAGTGTACAGC
L04473	TTTTTTCTGTGTTGGTTGCCAGTTTATAGT
M73969	TTCTTGCTTTGCTGGCTGCCCTACAACCTG
X65858	TTCTTGCTTTGCTGGCTGCCCTACAACCTG
S46665	TTCTTTATCTTCTGGCTGCCCTATCAGGTG
M60626	TTTTTTCTCTGCTGGTCCCATATCAGGTG

FIGURE 7

OLIGODEOXYNUCLEOTIDE SEQUENCE FOR 5' SIDE PRIMER

Primer T2A

GTCACCAACITGTTTCATCCTCAICCTG

C

AC

GT T

A

HUMGALAREC

ACCACCAACCTGTTTCATCCTCAACCTG

RATADRA1B

CCCACCAACTACTTTATCGTCAACCTG

HUMADRB1

ACCACCAACCTGTTTCATCCTCAACCTG

RABIL8RSB

GTCACCGACGTCTACCTGCTGAACCTG

HUMOPIODRE

GTCACCAACTCCTTCCTCGTGAACCTG

BTSKR

GTGACCAACTACTTCATCGTCAACCTG

HUMSRI2A

ATCACCAACATTTACATCCTCAACCTG

HUMSSTR3Y

GTCACCAACGTCTACATCCTCAACCTG

HUMGARE

GTCACCAACGCCTTCCTCCTCTCACTG

HUMCCKAR

GTCACCAACATCTTCCTCCTCTCCCTG

HUMSHTR

CCCTCCAACCTACCTGATCGTGTCCCTG

HUMD1B

ATGACCAACGTCTTCATCGTGTCTCTG

HUM5HT1E

CCTGCCAACTACCTAATCTGTTCTCTG

HUMD4C

CCCACCAACTCCTTCATCGTGAGCCTG

MMSERO

GCCACCAACTATTTTCCTGATGTCACTT

RATADRA1A

GTCACCAACTATTTTCATCGTGAACCTG

S57565

CTGACCAATTGCTTCATTGTGTCCCTG

FIGURE 8

COMPLEMENTARY OLIGODEOXYNUCLEOTIDE SEQUENCE TO 3' SIDE PRIMER

Complementary Sequence AACCCCTCITCTATTGCTTTITCICT
to Primer T7A T T C C C G G

HUMGALAREC	AATCCTATCATTTATGCATTTCTCTCT
RATA1ADREC	AACCCCATCGTCTATGCCTTCCGGATC
PIGA2R	AATCCTCTCTTTTATGGCTTTCTGGGG
RAT5HTRTC	AACCCTATCATCTACCCGCTCTTTATG
S58541	AACCCCATCATTTATGCCTTTAATGCT
HUMGRPR	AACCCCTTTGCCCTCTACCTGCTGAGC
MUSGRPBOM	AACCCCTTTGCTCTTTATCTGCTGAGC
RRVT1AIIR	AACCCTCTGTTCTACGGCTTTCTGGGG
HUMADRB1	AACCCCATCATCTACTGCCGCAGCCCC
HSHM4	AACCCCGTGTGCTATGCTCTGTGCAAC
HUMGARE	AACCCCTGGTCTACTGCTTCATGCAC
RATCCKAR	AACCCCATCATCTATTGCTTCATGAAC
S59749	AATCCCATGCTCTACACCTTCGCTGGC
HUMSST28A	AACCCCGTCCTCTACGGCTTCCTCTCG
RNGPROCR	AACCCCATCCTCTACGGCTTCCTCTCC
MUSSSRI1A	AACCCCATACTCTACGGCTTCCTGTCTG
HUMA1AADR	AACCCGCTCATCTACCCCTGTTCCAGC
S66181	AACCCGGTTCTCTACGCCTTCCTGGAC
HUMSSTR3Y	AACCCCATCCTTTATGGCTTCCTCTCC

FIGURE 9

OLIGODEOXYNUCLEOTIDE SEQUENCE FOR 5' SIDE PRIMER

Primer TM1-A2 TGITGGTTATIGGIGTTGTIGGIAA
 CC GC C G

MUSBB2R	TGGTGGTGGTGGTGGTGGTGGGCAA
BTSKR	TGGTGCTGGTGGCTGTGATGGGCAA
BOVEETBR	TGTTTCGTGCTGGGCATCATCGGAAA
HUMNEUYREC	TGATCATTCTTGGTGTCTCTGGAAA
MMSUBKREC	TGGTGCTGGTGGCTGTAACAGGCAA
HUMPG2R	TGTTTCATCTTCGGGGTGGTGGGCAA
HUMPIR	TGTTTCGTGGCCGGTGTGGTGGGCAA
HSU11053	TGTTTCGTGCTGGGCTTGGTGGGCAA
RRMC3RA	TGGTGATCCTGGCTGTGGTGAGGAA
HUMMR	TGGTTATCCTGGCCGTGGTCAGGAA
MUSGRPBOM	TCATCGTGATAGGTCTTATTGGCAA
RATCHOLREC	TCTTTCTGATGAGTGTTGGCGGAAA
RATCCKAR	TATTCCTTCTCAGTGTGCGGGGGAA

FIGURE 10

COMPLEMENTARY OLIGODEOXYNUCLEOTIDE SEQUENCE TO 3' SIDE PRIMER

Complementary Sequence	GCCAT	IACCITGGACAGATACCGAT
to Primer TM3-B2	A	T A C G A G

HUMCCKR	GCCATCGCACTGGAGCGGTACAG
HUMCCKBGR	GCCATCGCACTGGAGCGGTACAG
MMGMC5R	GCCATTGCGGTGGACAGGTACA
HUMV2R	GCCATGACGCTGGACCGCCACCG
RATNEURA	GCCATTGCAGTGGACAGGTA
DOGGSTRN	GCCATCGCCCTGGAGCGATACAG
RAT5HT5A	GCAATAGCTTTGGACCGCTACTGGT
MUSALP2ADA	GCCATTAGTCTGGACCGCTACTGGT
HUMADORA1X	GCAATTGCTGTGGACCGCTACC
HUMOPIODRE	GCCATCGCGGTGGACAGATACA
MUSGRPBOM	GCACTGTCAGCTGACAGGTACAAA
RATCCKAR	GCCATCTCTCTGGAGAGATATGG
HSTRHREC	GCCTTTACCATTGAGAGGTACATA

FIGURE 11

OLIGODEOXYNUCLEOTIDE SEQUENCE FOR 5' SIDE PRIMER

Primer TM3-C2	CATGGCCGTGGAGAGITACITGGC TT C C T A
HUMNK3R	CATTGCGGTGGACAGGTATATGGC
HSMRNAOXY	CATGTCCCTGGACCGCTGCCTGGC
S68242	CATATCGCTGGAGAGATACGGAGC
CFGPCR4	CATCGCTCTGGACAGGTACTGGGC
MMSUBPREC	TGGCCTTTGACAGATACATGGC
HUMOPIODRE	CATCGCGGTGGACAGATACATGGC
HUMGALAREC	ATGTCCGTGGACCGCTACGTGGC
HSS31G	CATTGCCCTGGACAGGTACTGGGC
HUMARB3A	CCTGGCCGTGGACCGCTACCTGGC
HUMHPR	CATGGCCGTGGAGCGCTGCCTGGC
RATCCKAR	CATCTCTCTGGAGAGATATGGCGC

FIGURE 12

COMPLEMENTARY OLIGODEOXYNUCLEOTIDE SEQUENCE TO 3' SIDE PRIMER

Complementary Sequence TTTGCCITCTGCTGGATCCCCAAC
to Primer TM6-E2 C G C G TT

HUMNEKAR	TTTGCCATCTGCTGGCTGCCCTAC
HUMSUBPRA	TTCGCCATCTGCTGGCTGCCCTTC
RATSKR	TTTGCCATCTGCTGGCTGCCCTAC
MUSGRPBOM	TTTGCCCTTCTGCTGGCTCCCCAAC
HUMOPIODRE	TTTGCCATCTGCTGGCTGCCCTA
HUMA2XXX	TTTGCCCTCTGCTGGCTGCCCCT
HUMADRBR	TTCACCCTCTGCTGGCTGCCCTTC
CFGPCR8	TTCGCCCCCTCTGTGGCTGCCCCT
HUMETSR	TTTGCCCTCTGCTGGCTTCCCCT
MMNPY1CDS	TTCGCCGTCTGCTGGCTGCCCCT
HSMRNOXY	TTCATCGTGTGCTGGACGCCTTTC
RATCCKAR	TTCTTCCTGTGCTGGATGCCCATC

FIGURE 13

OLIGODEOXYNUCLEOTIDE SEQUENCE FOR 5' SIDE PRIMER

Primer TM2F18

ARYYTIGCIITIGCNGAY

HUMTSHX	AACCTGGCCTTTGCGGAT
HUMNEKAR	AATCTGGCGCTGGCTGAC
HUMFMLP	AACCTGGCCGTGGCTGAC
HUMINTLEU8	AACCTAGCCTTGGCCGAC
HUMA1AADR	AACCTGGCCGTGGCCGAC
HUMIL8RA	AACCTGGCCTTGGCCGAC
HSDD2	AGCCTCGCAGTGGCCGAC
HUMANTIR	AATTTAGCACTGGCTGAC
HUMSOMAT	AACCTGGCCGTAGCCGAC
HUMEL4REC	AGCTTGGCTGTGGCTGAT
HSTRHREC	AGCCTGGCAGTAGCTGAT
HSU07882	AACCTGGCCTTAGCCGAT

(R = A or G, Y = C or T, N = A, C, G or T, and
I = Inosine)

FIGURE 14

COMPLEMENTARY OLIGODEOXYNUCLEOTIDE SEQUENCE TO 3' SIDE PRIMER

Complementary Sequence TTYNNNTNTGYTGGITICCI
to Primer TM6R21

HSBAR	TTCACCCTCTGCTGGCTGCCC
HUMNEKAR	TTTGCCATCTGCTGGCTGCCC
HUMETN1R	TTTGCTCTTTGCTGGTTCCCT
HUMHISH2R	TTCATCATCTGCTGGTTTCCC
HUMA1AADR	TTCGTGCTCTGCTGGTTCCCT
HUMIL8RA	TTCTGCTTTGCTGGCTGCCC
HUMNMBR	TTCATCTTCTGTTGGTTTCCCT
HUMNKIRX	TTGCCATCTGCTGGCTGCCC
HUMSUBPRA	TTGCCATCTGCTGGCTGCCC
HUM5HT1DA	TTTATCATCTGCTGGCTGCCC
HUMPFPR2A	TTCTTCATCTGTTGGTTTCCC
HSDD2	TTCATCATCTGCTGGCTGCCC
HUMNEUYREC	TTTGCACTCTGCTGGCTCCCT
HUM2XXX	TTTGCCCTCTGCTGGCTGCCC
HUMBK2A	TTCATCATCTGCTGGCTGCCC
HUMFMLPX	TTCTTCATCTGTTGGTTTCCC
HUMSSTR3X	TTCGTGCTCTGCTGGATGCCC
HUMCCKR	TTTTTTCTGTGTTGGTTGCCA
HSNEURA	TTTGTGGTCTGCTGGCTGCCC

(Y = C or T, N = A, C, G or T, and I = Inosine)

FIGURE 15

OLIGODEOXYNUCLEOTIDE SEQUENCE FOR 5' SIDE PRIMER

Primer S3A	GCCTGITIAIGATGAGTGTGGAIAGIT
	C G C TC C

HUMGALAREC	CCCTGGCCGCGATGTCCGTGGACCGCT
S70057	GCCTCGTGGCCATCGCACTGGAGCGGT
S67127	ACCTCTGCGCTCTTAGTGTTGACAGGT
S44866	GTCTATGTGCTCTGAGTATTGACAGAT
HUMC5AAR	TCCTGGCCACCATCAGCGCCGACCGCT
HUMANTIR	TACTCACGTGTCTCAGCATTGATCGAT
HUMBK2A	TCCTGATGCTGGTGAGCATCGACCGCT
HSNEURA	ACGTGGCCAGCCTGAGTGTGGAGCGCT
HUMGRPR	CACTCACGGCGCTCTCGGCAGACAGAT
HUMFSRS	GCCTGACAGTCATGAGCGTGGACCGCT
HUMIL8RA	TGTTGGCCTGCATCAGTGTGGACCGTT
HUMNEKAR	CCATGACCGCCATTGCTGCCGACAGGT

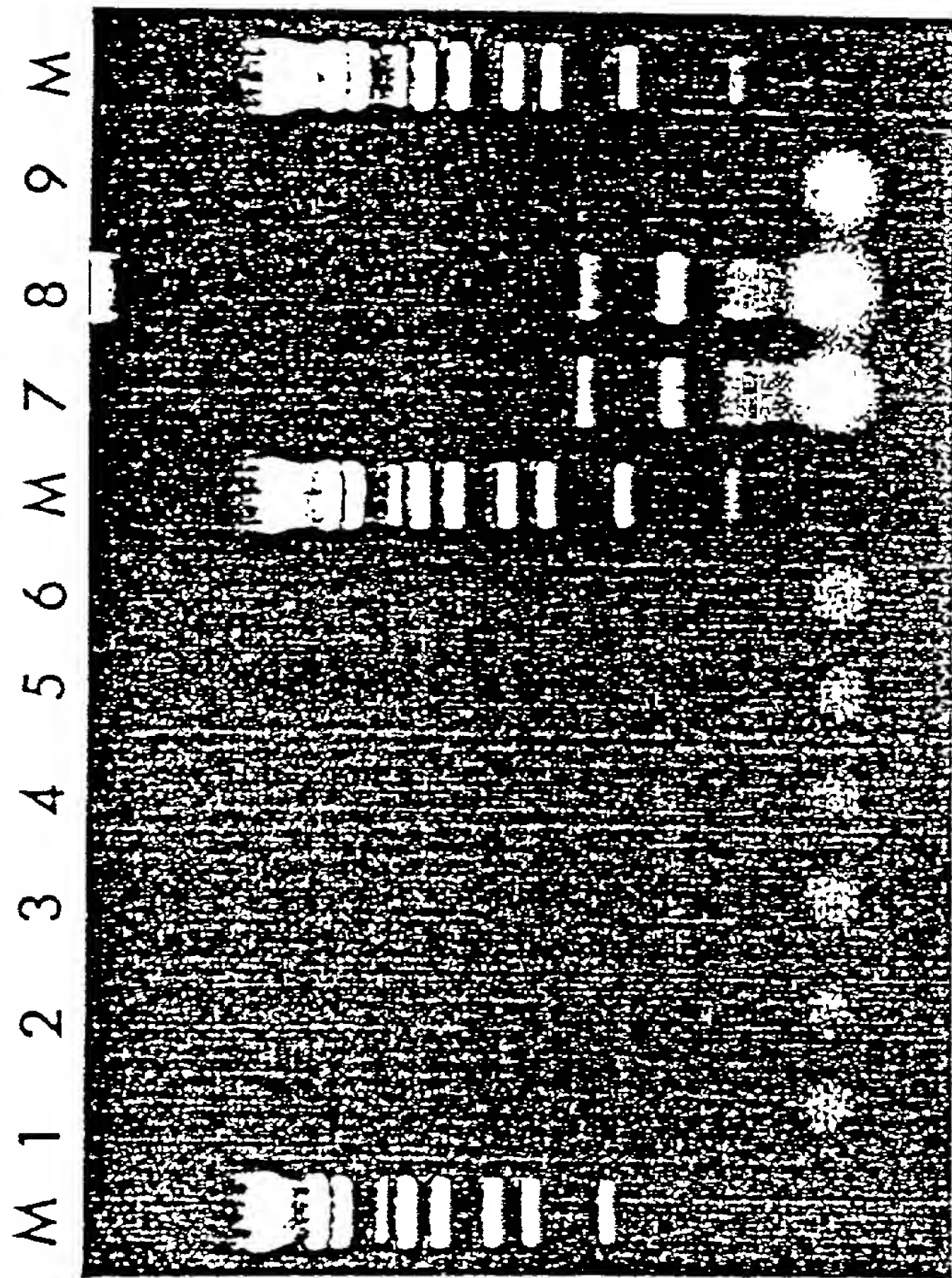
FIGURE 16

COMPLEMENTARY OLIGODEOXYNUCLEOTIDE SEQUENCE TO 3' SIDE PRIMER

Complementary Sequence	TGGITICCCTACCACITIAATCAICATC
to Primer S6A	T T GG GT

HUMGALAREC	TGGCTGCCGCACCACATCATCCATCTC
S70057	TGGTTGCCAGTTTATAGTGCCAACACG
S67127	TGGTTCCCTCTTCATTTAAGCCGTATA
S44866	TGGCTTCCCCTTCACCTCAGCAGGATT
HUMC5AAR	TGGTTGCCCTACCAGGTGACGGGGATA
HUMANTIR	TGGATTCCCCACCAAATATTCACTTTT
HUMBK2A	TGGCTGCCCTTCCAGATCAGCACCTTC
HSNEURA	TGGACTCCGTTCTCTATGACTTCTAC
HUMGRPR	TGGCTCCCCAATCATGTCATCTACCTG
HUMFSRS	TGGCTGCCCTTCTTCACCGTCAACATC
HUMIL8RA	TGGCTGCCCTACAACCTGGTCCTGCTG
HUMNEKAR	TGGCTGCCCTACCACCTCTACTTCATC

FIGURE 17



A58-T7-2	10	20	30	40	50
	GTGGGCATGGTGGGCAACCCCTGGTCA	TCTTCGTGATCCTTCGCTACGC			
HUMSOMAT	X:::	:::::::::	:::::::::	:::::::::	:::::::::
	GTGGGGCTGGTGGGCAACGCCCTGGTCA	TCTTCGTGATCCTTCGCTACGC			
	285	295	305	315	325
A58-T7-2	60	70	80	90	100
	CAAGATGAAGACGGCTACCAACATCTAC	CTGCTCAACCTGGCCGTAGCCG			
HUMSOMAT	:::::::::	:::::::::	:::::::::	:::::::::	:::::::::
	CAAGATGAAGACGGCTACCAACATCTAC	CTGCTCAACCTGGCCGTAGCCG			
	335	345	355	365	375
A58-T7-2	110	120	130	140	150
	ACGAGCTCTTCATGCTGAGCGTGCCCT	TTCGTGGCCTCGTCGGCCGCCCTG			
HUMSOMAT	:::::::::	:::::::::	:::::::::	:::::::::	:::::::::
	ACGAGCTCTTCATGCTGAGCGTGCCCT	TTCGTGGCCTCGTCGGCCGCCCTG			
	385	395	405	415	425
A58-T7-2	160	170	180	190	200
	CGCCACTGGCCCCTTCGGCTCCGTGCT	TGTCGCCGCCGGTGTCTCAGCGTCGA			
HUMSOMAT	:::::::::	:::::::::	:::::::::	:::::::::	:::::::::
	CGCCACTGGCCCCTTCGGCTCCGTGCT	TGTCGCCGCCGGTGTCTCAGCGTCGA			
	435	445	455	465	475
A58-T7-2	210	220	230	240	
	CGGCCCAACATGTTCACCACCGCTCT	TCTCTCACCCTGCTCAGCGT			
HUMSOMAT	:::::::::	:::::::::	:::::::::	:::::::::	:::::::::
	CGGCCCAACATGTTCACCACCGCTCT	TCTCTCACCCTGCTCAGCGT			
	485	495	505	515	

FIGURE 19

A58-SP6	10	20	30	40	50
	CAGTGTCCACACCCGGCCTGGTCGGCAGTCTTCGTGGTCTACACTTTCCT				
HUMSOMATA	X:::	:::	:::	:::	:::
	CAGTGGCCACACCCGGCCTGGTCGGCAGTCTTCGTGGTCTACACTTTCCT				
	706	716	726	736	746
A58-SP6	60	70	80	90	100
	GCTGGGCTTCCTGCTGTCCGTGCTGTCCATGGCCGTGTGCTACCTGCTCA				
HUMSOMATA	:::	:::	:::	:::	:::
	GCTGGGCTTCCTGCTGTCCCGTGTGTGGCCATGGCCGTGTGCTACCTGCTCA				
	756	766	776	786	796
A58-SP6	110	120	130	140	150
	TCGTGGCAAGATGCGCGCCGTGTCCCTGCGCGCTGGCTGGCAGCAGCGC				
HUMSOMATA	:::	:::	:::	:::	:::
	TCGTGGCAAGATGCGCGCCGTGTGGCCCTGCGCGCTGGCTGGCAGCAGCGC				
	806	816	826	836	846
A58-SP6	160	170	180	190	200
	AGGCGCTCGGAGAAGAAATCACCAGGCTGGTGTGATGGTGGTGGTGGT				
HUMSOMATA	:::	:::	:::	:::	:::
	AGGCGCTCGGAGAAGAAATCACCAGGCTGGTGTGATGGTGGTGGTGGT				
	856	866	876	886	896
A58-SP6	210	220			
	<u>CTTTGCCCTCTGCTGGTGTGCCCTCTCCAC</u>				
HUMSOMATA	:::	:::	:::	:::	X
	CTTTGTGCTCTGCTGGATGCCCTTCTCTAC				
	906	916			

	10	20	30	40	50
57-A-2	<u>GTGGGCATGCTGGGCAACCTCCTGGAAGGCAGTCGCCGAGGTGGCCGGTT</u>				
	X::: ::::: :: : ::				
HUMDRD5A	GTGGCGCTGCTGGTCATGC-CCTGGAAGGCAGTCGCCGAGGTGGCCGGTT				
	424	434	444	454	
	60	70	80	90	100
57-A-2	ACTGGCCCTTTGGAGCGTTCTGCGACGTCTGGGTGGCCTTCGACATCATG				
	::				
HUMDRD5A	ACTGGCCCTTTGGAGCGTTCTGCGACGTCTGGGTGGCCTTCGACATCATG				
	464	474	484	494	504
	110	120	130	140	150
57-A-2	TGCTCCACTGCCTCCATCCTGAACCTGTGCGTCATCAGCGTGGACCGCTA				
	::				
HUMDRD5A	TGCTCCACTGCCTCCATCCTGAACCTGTGCGTCATCAGCGTGGACCGCTA				
	514	524	534	544	554
	160	170	180	190	200
57-A-2	CTGGGCCATCTCCAGGCCCTTCCGCTACAAGCGCAAGATGACTCAGCGCA				
	::				
HUMDRD5A	CTGGGCCATCTCCAGGCCCTTCCGCTACAAGCGCAAGATGACTCAGCGCA				
	564	574	584	594	604
	210	220	230	240	250
57-A-2	TGGCCTTGGTCATGGTCGGCCTGGCATGGACCTTGTCCATCCTCATCTCC				
	::				
HUMDRD5A	TGGCCTTGGTCATGGTCGGCCTGGCATGGACCTTGTCCATCCTCATCTCC				
	614	624	634	644	654
	260	270	280	290	300
57-A-2	TTCATTCCGGTCCAGGTCAACTGGGACAGGGACCAGGCGGGCTCTTGGGG				
	:::::::::::::::::::: ::::: :::::::::::::: ::::::::::::::				
HUMDRD5A	TTCATTCCGGTCCAGCTCAACTGGCACAGGGACCAGGCGGCCTCTTGGGG				
	664	674	684	694	704
	310				
57-A-2	GGGGCTGGACCTGCCAAA				
	:::::::::::::::::::::X				
HUMDRD5A	CGGGCTGGACCTGCCAAA				
	714	724			

B54		10	20	30	40	50	
RNU04738		<u>GTGGGCATCGTGGGCAACATCC</u> TGGTCATAATTCGTGATCCTACGCTATGC					
	X:::	:	:	:	:	:	
	GTGGGCCCTGGTAGGAACGCCC	TGGTCATAATT	CGTGATCCTACGCTATGC				
	233	243	253	263	273		
B54		60	70	80	90	100	
RNU04738		CAAATGAAGACAGCCACCACAATCTACCTGCTCAACCTGGCCGTGCGCTG					
	:::	:	:	:	:	:	
	CAAATGAAGACAGCCACCACAATCTACCTGCTCAACCTGGCCGTGCGCTG						
	283	293	303	313	323		
B54		110	120	130	140	150	
RNU04738		ATGAGCTCTTCA TGCTCAGTGTGCCA TT TGTGGCC TCGGGCGCTGCCCTG					
	:::	:	:	:	:	:	
	ATGAGCTCTTCA TGCTCAGTGTGCCA TT TGTGGCC TCGGGCGCTGCCCTG						
	333	343	353	363	373		
B54		160	170	180			
RNU04738		CGCCACTGGCCGTT CGGGCGGTGCTGTGCCGC					
	:::	:	:	:	:	:	
	CGCCACTGGCCGTT CGGGCGGTGCTGTGCCGC						
	383	393	403				

FIGURE 22

5'		9	18	27	36	45	54
	GTG GGC ATG GTG GGC AAC GTC CTC CTG CTG GTG ATC GCG CGG GTG CGC CGG						
	Val Gly Met Val Gly Asn Val Leu Leu Val Val Ile Ala Arg Val Arg Arg						
		63	72	81	90	99	108
	CTG CAC AAC GTG ACG AAC TTC CTC CTC AAC GGC AAC CTG GCC TTC TCC GAC GTG CTC						
	Leu His Asn Val Thr Asn Phe Leu Ile Gly Asn Leu Ala Leu Ser Asp Val Leu						
	117	126	135	144	153	162	
	ATG TGC ACC GCC TGC GTG CCG CTC CCG CTC GGC TTC GAG CCA CCG GGC						
	Met Cys Thr Ala Cys Val Pro Leu Thr Leu Ala Tyr Ala Phe Glu Pro Arg Gly						
	171	180	189	198	207	216	
	TGG GTG TTC GGC GGC GTC CTC TGC CAC CAC CTG GTC TTC CTG CAG CCG GTC ACC						
	Trp Val Phe Gly Gly Leu Cys His Leu Val Phe Phe Leu Gln Pro Val Thr						
	225	234	243	252	261	270	
	GTC TAT GTG TCG GTG TTC ACG CTC CTC ACC ACC ATC GAA GTG GAC CCG TAC GTC GGT						
	Val Tyr Val Ser Val Phe Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr						
	279	288	297				
	GCT GGT GCA CCC GCT GAG GCG GCG CAT 3'						
	Ala Gly Ala Pro Ala Glu Ala Gly His						

5'		9	18	27	36	45	54						
GGC	CTG	CTG	GTC	ACC	TAC	CTG	CTC	ATC	GTC	CTG	TCT	TAC	
---	---	---	---	---	---	---	---	---	---	---	---	---	
Gly	Leu	Leu	Val	Thr	-Tyr	Leu	Pro	Leu	Leu	Val	Ile	Ser	Tyr
		63	72	81	90	99	108						
GTC	CGG	GTG	TCA	GTG	AAG	CTC	CGC	AAC	CCG	GTG	CCG	GTC	CAG
---	---	---	---	---	---	---	---	---	---	---	---	---	---
Val	Arg	Val	Ser	Val	Lys	Leu	Arg	Asn	Pro	Val	Pro	Val	Gln
		117	126	135	144	153	162						
AGC	CAG	GCC	GAC	TGG	GAC	CGC	GCT	CGC	CGC	ACC	TTC	TTC	GTG
---	---	---	---	---	---	---	---	---	---	---	---	---	---
Ser	Gln	Ala	Asp	Trp	Asp	Arg	Ala	Arg	Arg	Arg	Thr	Phe	Leu
		171	180	189	198	3'							
GTG	GTC	GTG	GTG	GTG	TTT	GCC	ATC	TGC	TGG	TTC	TAC	TAC	Tyr
---	---	---	---	---	---	---	---	---	---	---	---	---	---
Val	Val	Val	Val	Val	Phe	Ala	Ile	Cys	Trp	Leu	Pro	Tyr	Tyr

at a very low level in the early 1970s, but it has since risen sharply to a level of about 2.5 in the early 1980s.

FIGURE 24

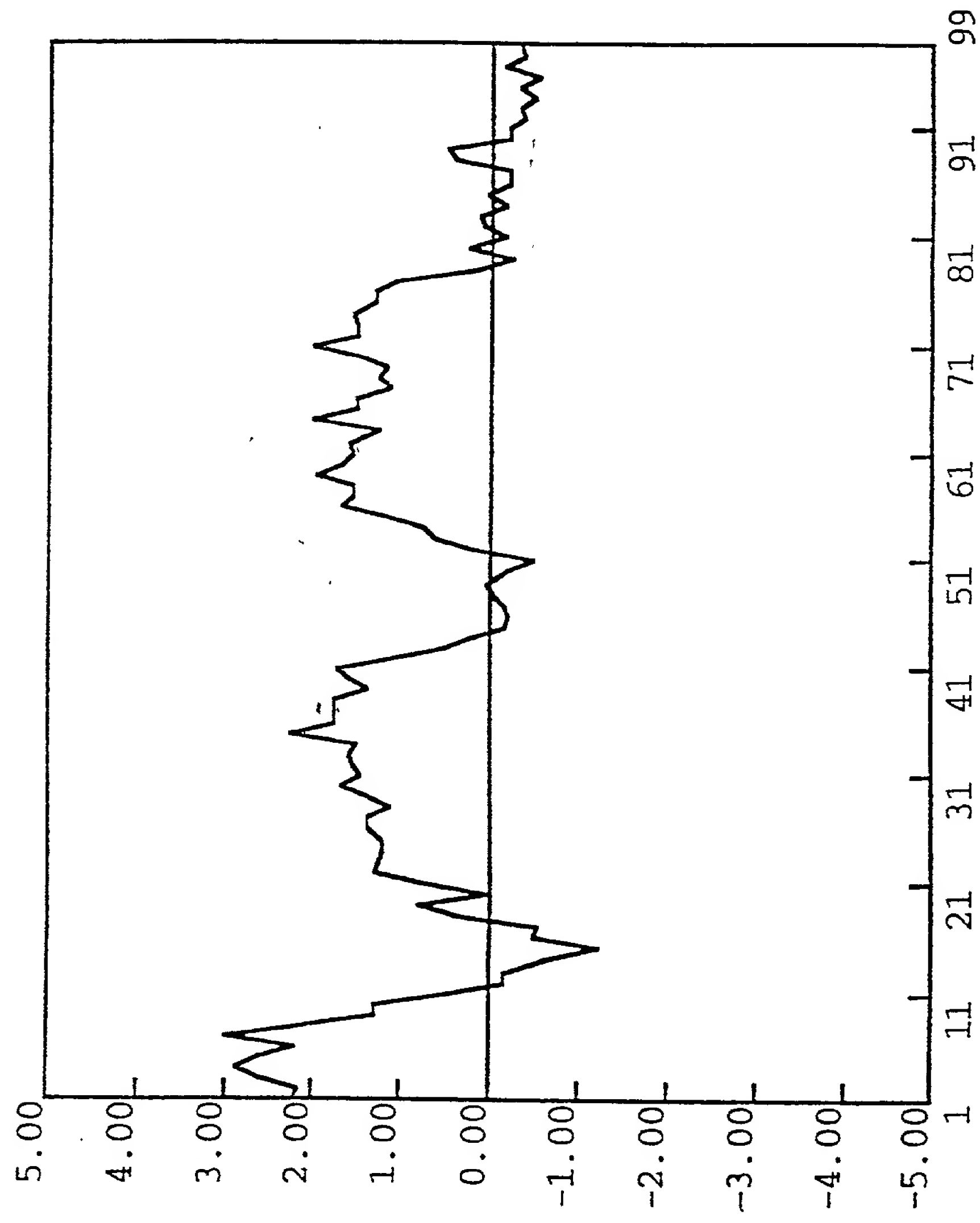


FIGURE 25

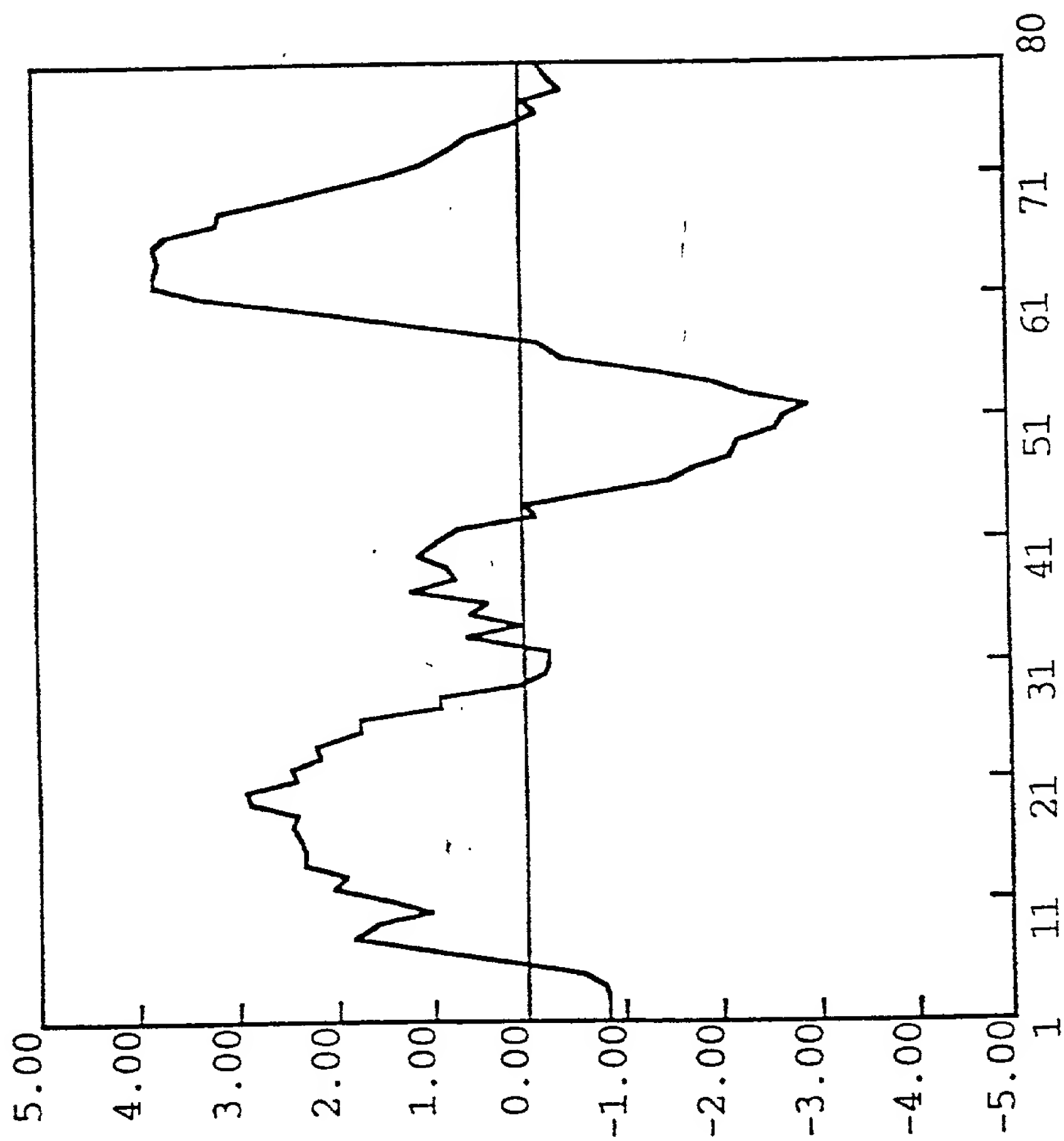


FIGURE 26

	10	20	30	40	50		
p19P2	1	VGMVGNVLLV	LVTARVRRLLH	NVTNFLIGNL	ALSDVLMCTA	CVPLTLAYAF	50
S12863	1	LGVSGNLALI	IIILKQKEMR	NVTNILLIVNL	SFSDLLVAVM	CLPFTFVYTL	50
	60	70	80	90	100		
p19P2	51	EPRGWVFGGG	LCHLVFFLQIP	VTVYVSVFTL	TTTEVDRYVG	AGAPAEAGH	100
S12863	51	MDH-WVFGET	MCKLNPEVQC	VSITVSI ESL	VLI AVERHQL	IINPRGWRPN	100
	110	120	130	140	150		
p19P2	101						150
S12863	101	NRHAYIGITV	IWVLAVASSL	PFVIYQILTD	EPFQNVSLAA	FKDKYVCFDK	150
	160	170	180	190	200		
p19P2	151	GLLLV	TYLLPLLVIL	LS-----Y	VRSVKLRNPV	VPVCVTQSQA	200
S12863	151	FPSDSHRLSY	TTLLLVLLQYF	GPLCFIFICY	FKIYIRLKRR	NNMMDKIRDS	200
	210	220	230	240	250		
p19P2	201	DWDRARRRRT	FCLLVVVVVV	FAICWLPYY	250
S12863	201	KYRSSETKRI	NVMLLSIVVA	FAVCWLPRLT	250

1976	1977	1978	1979	1980
1981	1982	1983	1984	1985
1986	1987	1988	1989	1990
1991	1992	1993	1994	1995
1996	1997	1998	1999	2000
2001	2002	2003	2004	2005
2006	2007	2008	2009	2010
2011	2012	2013	2014	2015
2016	2017	2018	2019	2020
2021	2022	2023	2024	2025
2026	2027	2028	2029	2030
2031	2032	2033	2034	2035
2036	2037	2038	2039	2040
2041	2042	2043	2044	2045
2046	2047	2048	2049	2050
2051	2052	2053	2054	2055
2056	2057	2058	2059	2060
2061	2062	2063	2064	2065
2066	2067	2068	2069	2070
2071	2072	2073	2074	2075
2076	2077	2078	2079	2080
2081	2082	2083	2084	2085
2086	2087	2088	2089	2090
2091	2092	2093	2094	2095
2096	2097	2098	2099	2100
2101	2102	2103	2104	2105
2106	2107	2108	2109	2110
2111	2112	2113	2114	2115
2116	2117	2118	2119	2120
2121	2122	2123	2124	2125
2126	2127	2128	2129	2130
2131	2132	2133	2134	2135
2136	2137	2138	2139	2140
2141	2142	2143	2144	2145
2146	2147	2148	2149	2150
2151	2152	2153	2154	2155
2156	2157	2158	2159	2160
2161	2162	2163	2164	2165
2166	2167	2168	2169	2170
2171	2172	2173	2174	2175
2176	2177	2178	2179	2180
2181	2182	2183	2184	2185
2186	2187	2188	2189	2190
2191	2192	2193	2194	2195
2196	2197	2198	2199	2200
2201	2202	2203	2204	2205
2206	2207	2208	2209	2210
2211	2212	2213	2214	2215
2216	2217	2218	2219	2220
2221	2222	2223	2224	2225
2226	2227	2228	2229	2230
2231	2232	2233	2234	2235
2236	2237	2238	2239	2240
2241	2242	2243	2244	2245
2246	2247	2248	2249	2250
2251	2252	2253	2254	2255
2256	2257	2258	2259	2260
2261	2262	2263	2264	2265
2266	2267	2268	2269	2270
2271	2272	2273	2274	2275
2276	2277	2278	2279	2280
2281	2282	2283	2284	2285
2286	2287	2288	2289	2290
2291	2292	2293	2294	2295
2296	2297	2298	2299	2300
2301	2302	2303	2304	2305
2306	2307	2308	2309	2310
2311	2312	2313	2314	2315
2316	2317	2318	2319	2320
2321	2322	2323	2324	2325
2326	2327	2328	2329	2330
2331	2332	2333	2334	2335
2336	2337	2338	2339	2340
2341	2342	2343	2344	2345
2346	2347	2348	2349	2350
2351	2352	2353	2354	2355
2356	2357	2358	2359	2360
2361	2362	2363	2364	2365
2366	2367	2368	2369	2370
2371	2372	2373	2374	2375
2376	2377	2378	2379	2380
2381	2382	2383	2384	

[illegible]

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FIGURE 28

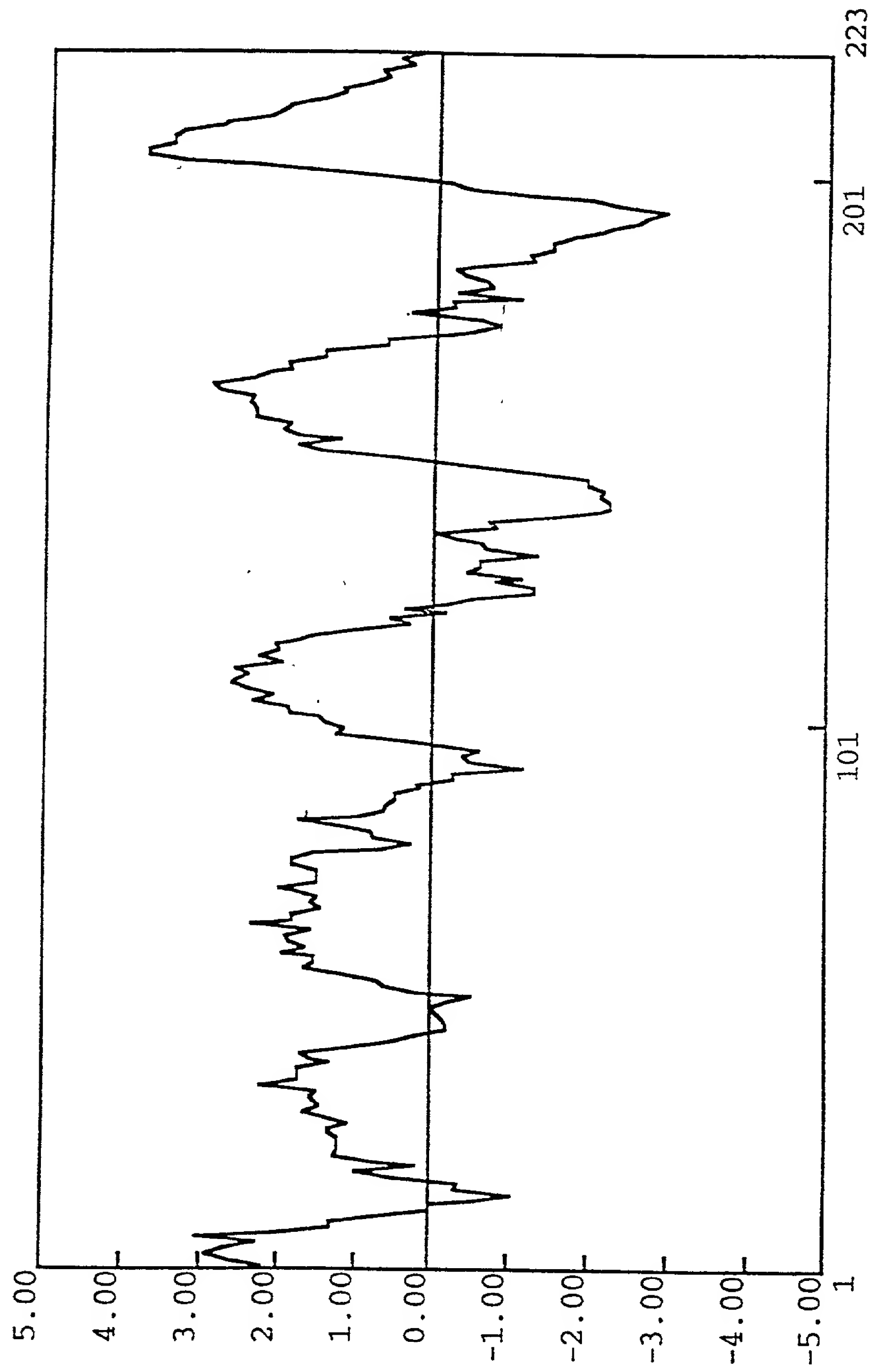


FIGURE 29

5'	10	19	28	37	46	55
GTG GGC ATG CTG GGC AAC GCC CTG	GTG CAT GTC ATC TTC AAG AAC CAG CGA	Val Cys His Val Ile Phe Lys Asn Gln Arg				
64	73	82	91	100	109	
ATG CAC TCG GCC ACC AGC CTC TTC ATC GTC AAC CTG GCA GTT GCC GAC ATA ATG						
Met His Ser Ala Thr Ser Leu Phe Ile Val Asn Leu Ala Val Ala Asp Ile Met						
118	127	136	145	154	163	
ATC ACG CTG CTC AAC ACC CCC TTC ACT TTG GTT CGC TTT GTG AAC ACC ACA TGG						
Ile Thr Leu Leu Asn Thr Pro Phe Thr Leu Val Arg Phe Val Asn Ser Thr Trp						
172	181	190	199	208	217	
ATA TTT GGG AAG GGC ATG TGC CAT GTC AGC CGC TTT GCC CAG TAC TGC TCA CTG						
Ile Phe Gly Lys Gly Met Cys His Val Ser Arg Phe Ala Gln Tyr Cys Ser Leu						
226	235					
CAC GTC TCA GCA CTG ACA 3'						
His Val Ser Ala Leu Thr						

5'	9	18	27	36	45	54
GAG CCA GCT GAC CTC TTC TGG AAG AAC CTG GAC TTG CCC ACC TTC ATC CTG CTC						
Glu Pro Ala Asp Leu Phe Trp Lys Asn Leu Asp Leu Pro Thr Phe Ile Leu Leu						
	63	72	81	90	99	108
AAC ATC CTG CCC CTC CTC ATC ATC TCT GTG GCC TAC GTT CGT GTG ACC AAG AAA						
Asn Ile Leu Pro Leu Leu Ile Ile Ser Val Ala Tyr Val Arg Val Thr Lys Lys						
	117	126	135	144	153	162
CTG TGG CTG TGT AAT ATG ATT GTC GAT GTG ACC ACA GAG CAG TAC TTT GCC CTG						
Leu Trp Leu Cys Asn Met Ile Val Asp Val Thr Thr Glu Gln Tyr Phe Ala Leu						
	171	180	189	198	207	216
CGG CCC AAA AAG AAG ACC ATC AAG ATG ATG TTG ATG CTG GTG GTA GTC CTC TTT						
Arg Pro Lys Lys Lys Lys Thr Ile Lys Met Leu Met Leu Val Val Leu Phe						
	225	234				
GCC CTC TGC TGG TGG CCT CTC GAC 3'						
Ala Leu Cys Trp Leu Pro Leu Asp						

31 1 7 9

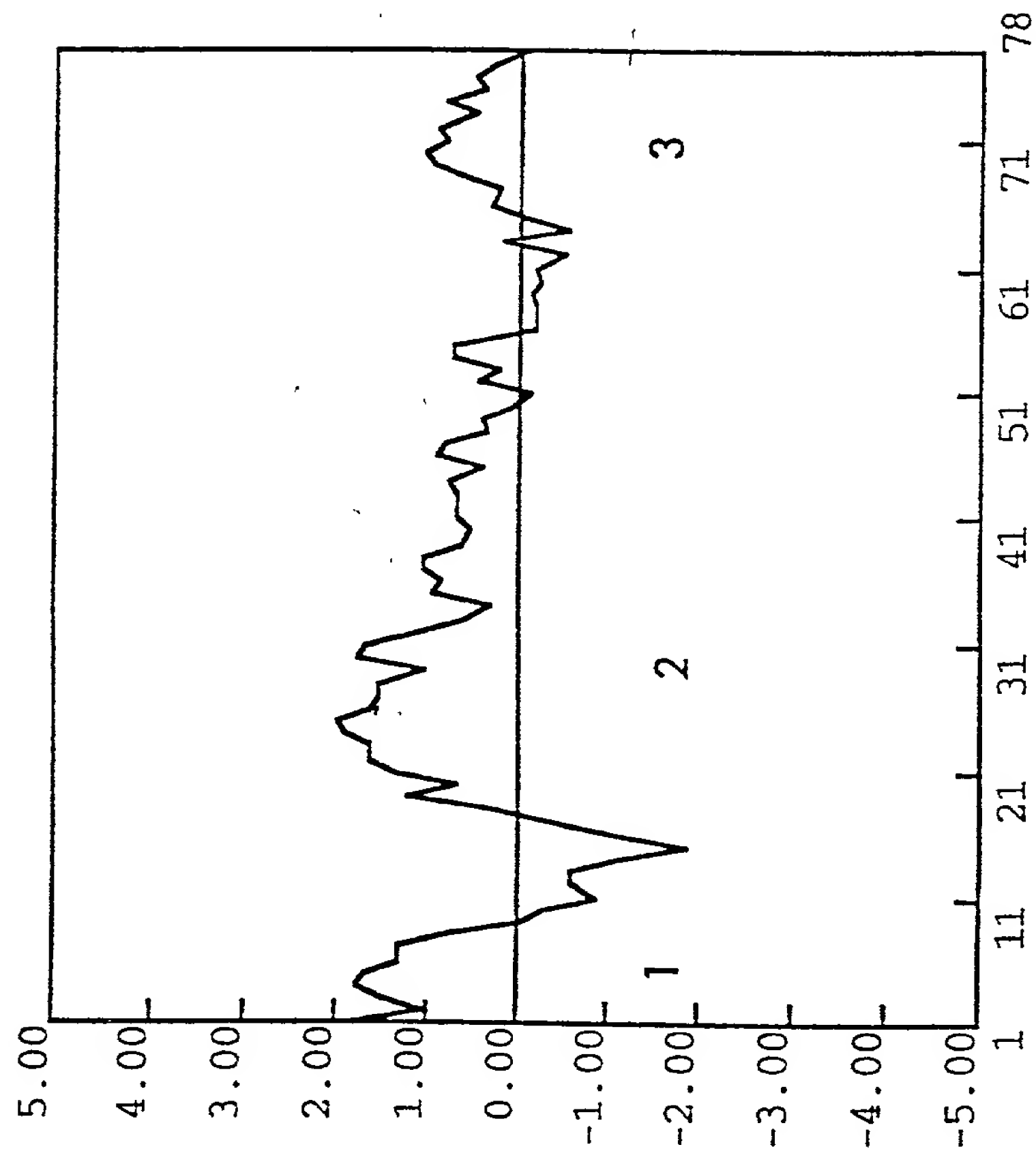
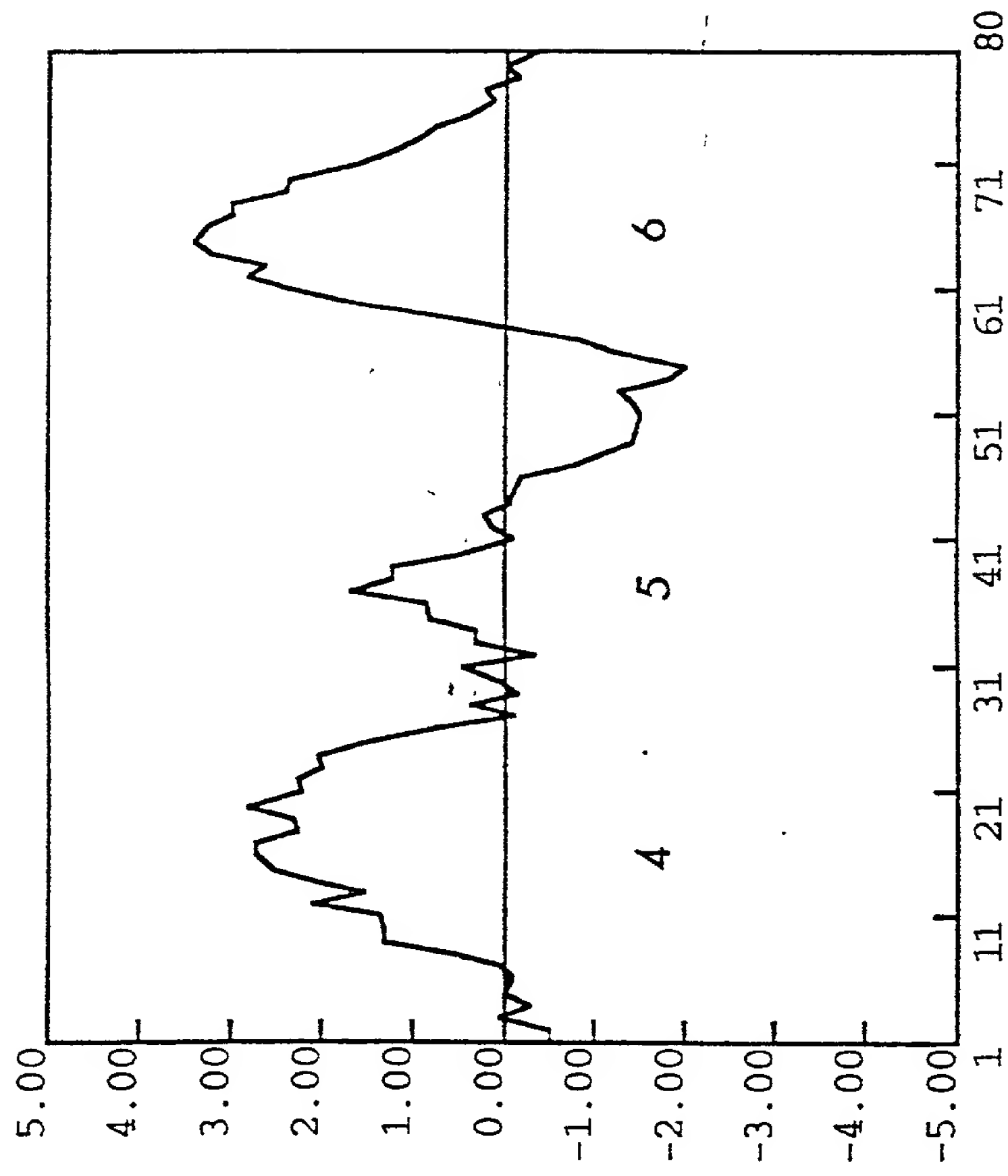


FIGURE 32



	10	20	30	40	50
p63A2	1 VCHVTEKNQR	MHSATSLFTV	NLAVADIMTT	LILNTEFFLVR	FVNSTWIFGK
P30731	1 VCHVTEKNQR	MHSATSLFTV	NLAVADIMTT	LILNTEFFLVR	FVNSTWVFGK
	60	70	80	90	100
p63A2	51 GMCHVSRFAQ	YCSLHVSALT			
P30731	51 GMCHVSRFAQ	YCSLHVSALT	LTAIAVDRHQ	VIMHPLKPRI	SITKGVITYIA
	110	120	130	140	150
p63A2	101 VIWVMATFFS	LPHAICQKLF	TFKYSEDIVR	SLCLPDFPEP	ADLEFWKYLDEL
P30731	101 VIWVMATFFS	LPHAICQKLF	TFKYSEDIVR	SLCLPDFPEP	ADLEFWKYLDL
	160	170	180	190	200
p63A2	151 PTFILINILP	LLIISVAVVR	VTKKLWLCNM	IVDVTTTEQYF	ALRPKKKKTTI
P30731	151 ATFFILLYLLP	LFIIISVAYAR	VAKKLWLCNT	IGDVTTTEQYL	ALRPKKKKTTV
	210	220	230	240	250
p63A2	201 KMMLLVVVVL				
P30731	201 KMMLLVVVVL				

FIGURE 34

1	CATCGTCAAGCAGATGAAGATCATCCACGAGGATGGCTACTCCGAGGGCCAGCAGAAATT	60
1		1
61	CTGCCCCCTTCTTCCCGCGAGTGCTTTCCCGCTCTCCAAACCCCACTCCCAGGTGGCCATG	120
1		Met 1
121	GCCTCATCGACCACTCGGGGCCCCAGGGTTTCTGACTTATTTCTGGGCTGCCGCCGGCG	180
1	AlaSerSerThrThrArgGlyProArgValSerAspLeuPheSerGlyLeuProProAla	21
181	GTCACAACCTCCCGCCAACCAGAGCGCAGAGGGCTCGGGCGGCAACGGGTGGTGGCTGGC	240
21	ValThrThrProAlaAsnGlnSerAlaGluAlaSerAlaGlyAsnGlySerValAlaGly	41
241	GCGGACGCTCCAGCCGTACGCCCTTCCAGAGCCTGCAGCTGGTGCATCAGCTGAAGGGG	300
41	AlaAspAlaProAlaValThrProPheGlnSerLeuGlnLeuValHisGlnLeuLysGly	61
301	CTGATCGTGCTGCTCTACAGCGTGGTGGTGGTGGGCTGGTGGGCAACTGCCTGCTG	360
61	LeuIleValLeuLeuTyrSerValValValValValGlyLeuValGlyAsnCysLeuLeu	81
361	GTGCTGGTGATCGCGCGGGTGGCGGGCTGCACAACGTGACGAACTTCCTCATCGGCAAC	420
81	ValLeuValIleAlaArgValArgArgLeuHisAsnValThrAsnPheLeuIleGlyAsn	101
421	CTGGCCTTGTCCGACGTGCTCATGTGCACCGCCTGCGTGGCGCTCAGCTGGCCTATGCC	480
101	LeuAlaLeuSerAspValLeuMetCysThrAlaCysValProLeuThrLeuAlaTyrAla	121
481	TTGAGCCACGCGGCTGGGTGTTCGGCGCGCGCCTGTGCCACCTGGTCTTCTTCCTGCAG	540
121	PheGluProArgGlyTrpValPheGlyGlyGlyLeuCysHisLeuValPhePheLeuGln	141
541	CCGGTCACCGTCTATGTGTGGTGTTCACGCTCACCACCATCGCAGTGGACCGCTACGTC	600
141	ProValThrValTyrValSerValPheThrLeuThrThrIleAlaValAspArgTyrVal	161
601	GTGCTGGTGACCCCGCTGAGGCGGCGCATCTCGCTGCGCCTCAGCGCCTACGCTGTGCTG	660
161	ValLeuValHisProLeuArgArgArgIleSerLeuArgLeuSerAlaTyrAlaValLeu	181
661	GCCATCTGGGCGCTGTCCGCGGTGCTGGCGCTGCCCGCGCGCGTGCACACCTATCACGTG	720
181	AlaIleTrpAlaLeuSerAlaValLeuAlaLeuProAlaAlaValHisThrTyrHisVal	201
721	GAGCTCAAGCCGCACGACGTGCGCCTCTGCGAGGAGTTCTGGGGCTCCCAGGAGCGCCAG	780
201	GluLeuLysProHisAspValArgLeuCysGluGluPheTrpGlySerGlnGluArgGln	221
781	CGCCAGCTCTACGCCTGGGGGCTGCTGCTGGTCACCTACCTGCTCCCTCTGCTGGTCATC	840
221	ArgGlnLeuTyrAlaTrpGlyLeuLeuLeuValThrTyrLeuLeuProLeuLeuValIle	241
841	CTCCTGTCTTACGTCCGGGTGTCACTGAAGCTCCGCAACCGGTGGTGGCGGGCTGCGTG	900
241	LeuLeuSerTyrValArgValSerValLysLeuArgAsnArgValValProGlyCysVal	261
901	ACCCAGAGCCAGGCGGACTGGGACCGCGCTCGGCGCGGCGCACCTTCTGCTTGCTGGTG	960
261	ThrGlnSerGlnAlaAspTrpAspArgAlaArgArgArgThrPheCysLeuLeuVal	281
961	GTGGTGGTGGTGGTGTTCGCCGTCTGCTGGCTGCCGCTGCAGCTCTTCAACCTGCTGCGG	1020
281	ValValValValValPheAlaValCysTrpLeuProLeuHisValPheAsnLeuLeuArg	301
1021	GACCTCGACCCCCACGCCATCGACCCCTACGCCCTTGGGCTGGTGCAGCTGCTCTGCCAC	1080
301	AspLeuAspProHisAlaIleAspProTyrAlaPheGlyLeuValGlnLeuLeuCysHis	321
1081	TGGCTCGCCATGAGTTCCGGCCTGCTACAACCCCTTCATCTACGCTGGCTGCACGACAGC	1140
321	TrpLeuAlaMetSerSerAlaCysTyrAsnProPheIleTyrAlaTrpLeuHisAspSer	341
1141	TTCCGCGAGGAGCTGCGCAAACTGTTGGTGGCTTGGCCCCGCAAGATAGCCCCCATGGC	1200
341	PheArgGluGluLeuArgLysLeuLeuValAlaTrpProArgLysIleAlaProHisGly	361
1201	CAGAATATGACCGTCAGCGTGGTCATCTGATGCCACTTAGCCAGGCCTTGGTCAAGGAGC	1260
361	GlnAsnMetThrValSerValValIle***	371
1261	TCCACTTCAACTGGCCTCCTAGGGCACCCTCGAGGTCAATCTGGTGCTTATTCTCAGCA	1320
371		371
1321	CCAGAGCTAGC	1331
371		371

FIGURE 35

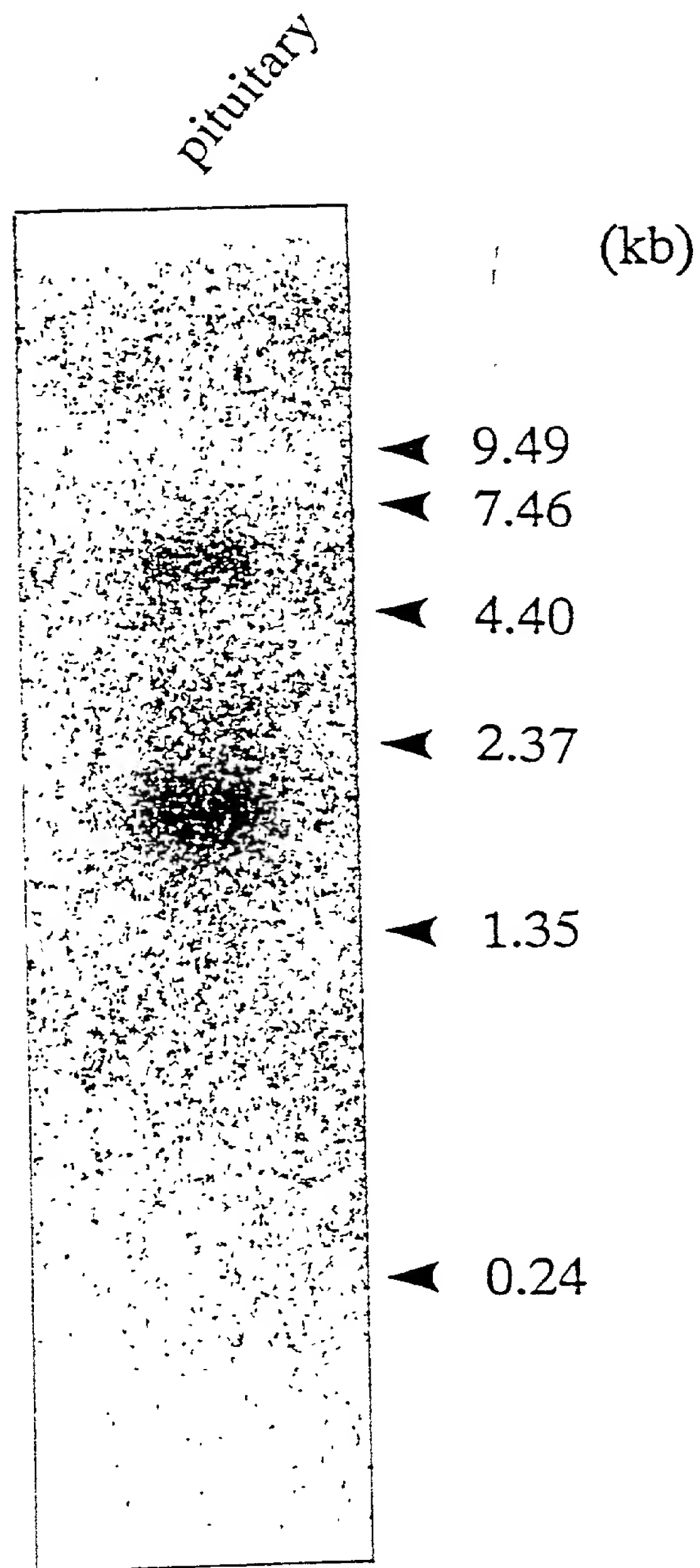
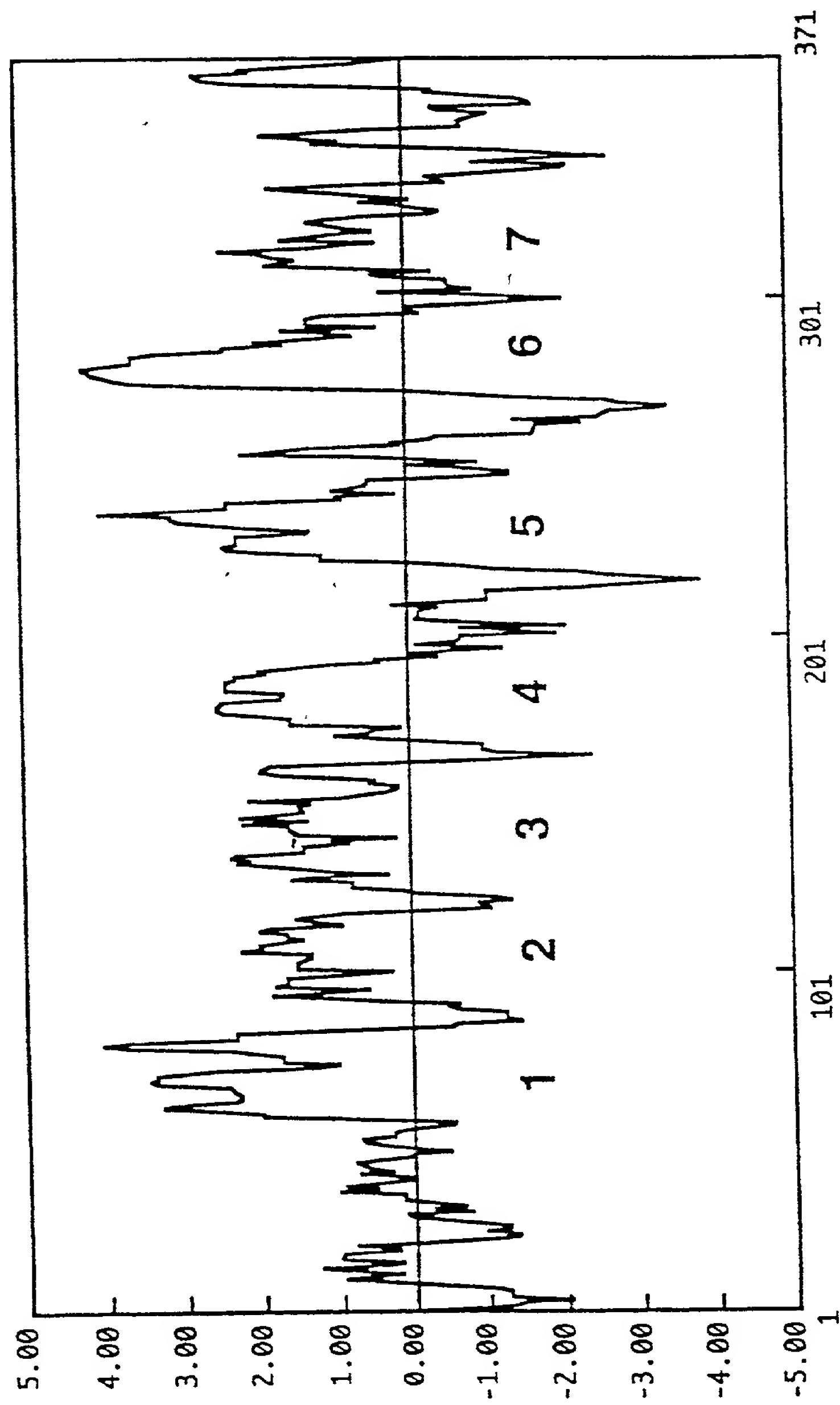
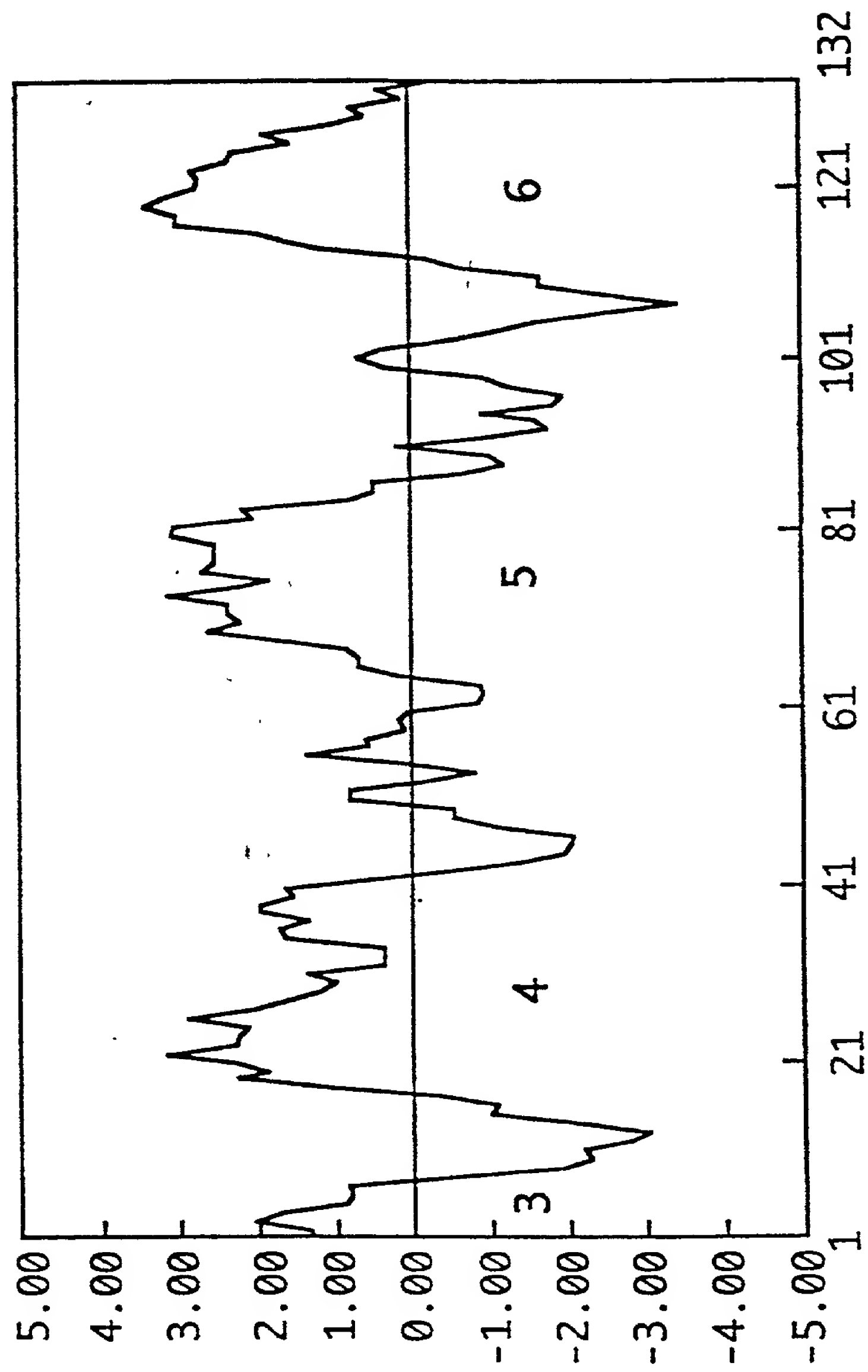


FIGURE 36



[illegible]

FIGURE 38



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FIGURE 39

p3H2-17	1	VGLVGNILAS	WHKRGGRRAA	WVVCVVWLA	VTAAQCLPTAV	FAATG QRN-	50
p34996	1	RYTG VVHP LK	SLGR LKKKNA	VYV SSLVWAL	VVA V AP L F	YSGTGVRRN-	50
A46226	1	RYLAVVHP TR	SARWRTAPVA	RTVSA AVWVA	SAVVVL PVVV	F--SGVPRG-	50
JN0605	1	RYVAVVHP LR	AATY RRPSVA	KL NLGVWLA	SL L V L P A I	FADTRPARGG	50
S28787	1	RYLA IVHATN	SQKPR KL LAE	KVVYVGVWLP	AVLLT I RDLI	FAD I KEVDE-	50
p3H2-17	51	RTV-CYDL--	SPP LSTRYL	PYGM ALT VIG	FL P F I A L A	CYCRMARR C	100
p34996	51	KT ITCYDT--	TAD E Y L RSYF	VYS M C T TVFM	FC P F I V L G	CYGL V K A L I	100
A46226	51	MST-CHMQWP	EPAA AWRAGF	I I Y --TAA L G	FFG P L L V I C L	CYLL V V K V R	100
JN0605	51	QAVACNLQWP	HPAWS AVFVV	YTF --L L G	FL P V L A I G L	CYLL V G K V R	100
S28787	51	RYI-CDRF--	YPSD LWL VVF	QFQ--H I V V G	L L P G I V L S	CYC I I S K L S	100
p3H2-17	101	RQD GPA-GPV	AQE-RRS--K	AARMA VVVAA	VFA L C W L P L Y	150
p34996	101	YK D LDN-SPL	--RR--K	S I Y L V I I V L T	VFAV S Y L P F H	150
A46226	101	SAGR RVWAP S	CQRRRR SERR	VTRM VVAVVA	L F V L C W M P F Y	150
JN0605	101	AVAL RA--G	WQQR RRSEK K	I T R L V L M V V V	V F V L C W M P F Y	150
S28787	101	HSK G-----	YQKR-----K	A L K T T V I L I L	T H F A C W L P Y Y	150

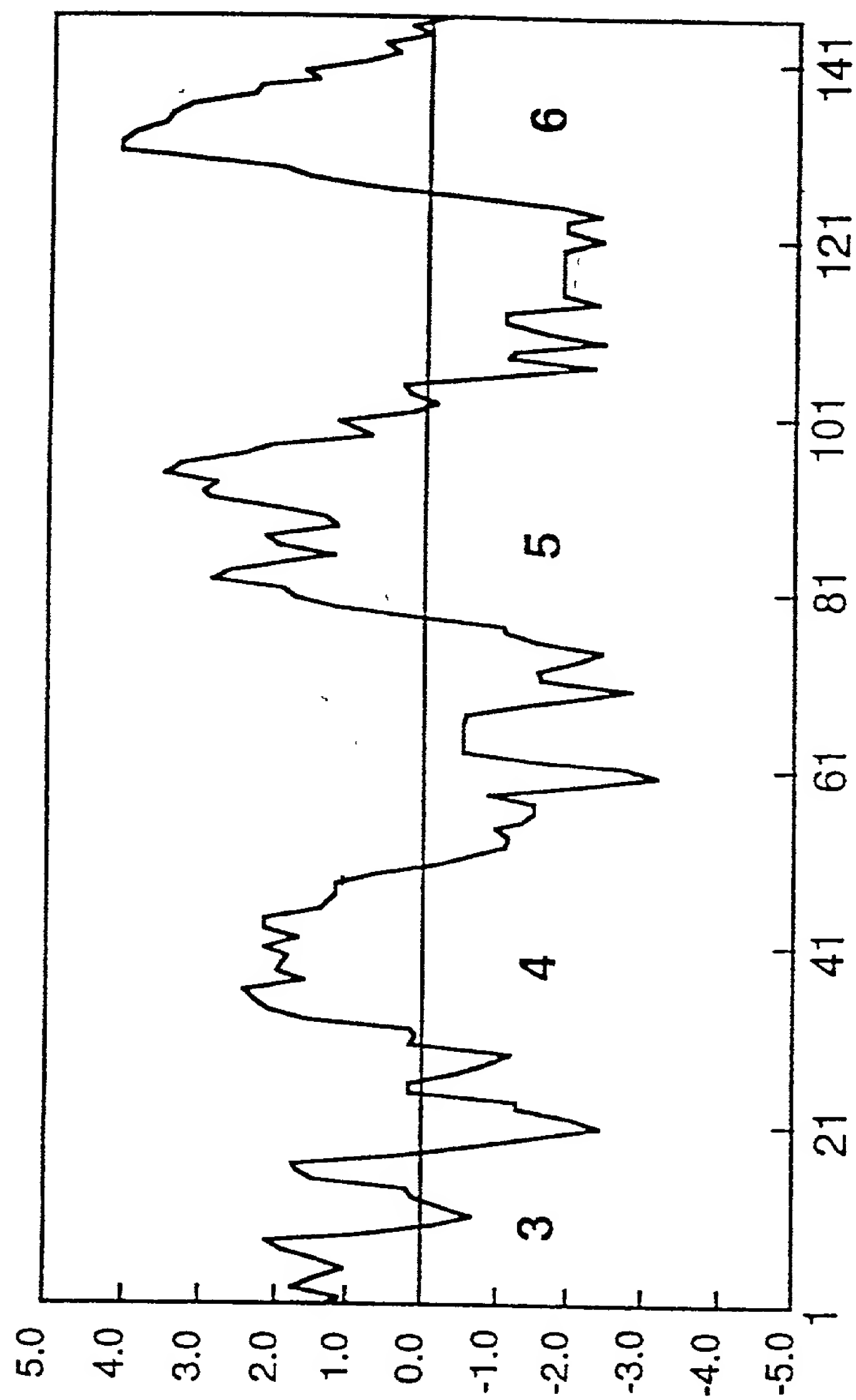
FIGURE 40

5'	GTG	GGC	CTG	GTG	GGC	AAC	TTC	CTG	GCC	GCG	ATG	TCT	GTG	GAT	CGC	TAC	GTG	GCC
	Val	Gly	Leu	Val	Gly	Asn	Phe	Leu	Ala	Ala	Met	Ser	Val	Asp	Arg	Tyr	Val	Ala
	ATT	GTG	CAC	TCG	CGG	CGC	TCC	TCC	TCC	CTC	AGG	GTG	TCC	CGC	AAC	GCA	CTG	CTG
	Ile	Val	His	Ser	Arg	Arg	Ser	Ser	Ser	Leu	Arg	Val	Ser	Arg	Asn	Ala	Leu	Leu
	GGC	GTG	GGC	TTC	ATC	TGG	GCG	CTG	TCC	ATC	GCC	ATG	GCC	TCG	CCG	GTG	GCC	TAC
	Gly	Val	Gly	Phe	Ile	Trp	Ala	Leu	Ser	Ile	Ala	Met	Ala	Ser	Pro	Val	Ala	Tyr
	CAC	CAG	CGT	CTT	TTC	CAT	CGG	GAC	AGC	AAC	CAG	ACC	TTC	TGC	TGG	GAG	CAG	TGG
	His	Gln	Arg	Leu	Phe	His	Arg	Asp	Ser	Asn	Gln	Thr	Phe	Cys	Trp	Glu	Gln	Trp
	CCC	AAC	AAG	CTC	CAC	AAG	AAG	GCT	TAC	GTG	GTG	TGC	ACT	TTC	GTC	TTT	GGG	TAC
	Pro	Asn	Lys	Leu	His	Lys	Lys	Ala	Tyr	Val	Val	Cys	Thr	Phe	Val	Phe	Gly	Tyr
	CTT	CTG	CCC	TTA	CTG	CTC	ATC	TGC	TTT	TGC	TAT	GCC	AAG	GTC	CTT	AAT	CAT	CTG
	Leu	Leu	Pro	Leu	Leu	Leu	Ile	Cys	Phe	Cys	Tyr	Ala	Lys	Val	Leu	Asn	His	Leu
	CAT	AAA	AAG	CTG	AAA	AAC	ATG	TCA	AAA	AAG	TCT	GAA	GCA	TCC	AAG	AAA	AAG	ACT
	His	Lys	Lys	Leu	Lys	Asn	Met	Ser	Lys	Lys	Ser	Glu	Ala	Ser	Lys	Lys	Lys	Thr
	GCA	CAG	ACC	GTC	CTG	GTG	GTC	GTT	GTA	GTA	TTT	GCC	CTC	TGC	TGG	CTG	CCT	TTC
	Ala	Gln	Thr	Val	Leu	Val	Val	Val	Val	Val	Phe	Ala	Leu	Cys	Trp	Leu	Pro	Phe

TAC 3'

Tyr

FIGURE 41



42 2 7 9

FIGURE 42

p3H2-34	1	VGLVGNELAA	MSVDRYVAIV	HSRRSSSLRV	SRNALGSGVF	IWALSAMAS	50
JN0605	1	MFTSVEGFTV	LSVDRYVAIV	HPLRAATYRR	PSVAKLINIG	VMLA SLIVIL	50
B41795	1	QFTSVEGFTV	MSLDRYVAIV	HPLKSAKWRR	PRTAKMITMA	VMGV SLIVIL	50
A39297	1	MFTSIYCEITV	LSVDRYVAIV	HPLKAARYRR	PIWAKVWNIG	VMVLSLIVIL	50
p3H2-34	51	PVA-YHORLF	HRDSNQTF	EQWPNKLHK-	-KAYVVCITFV	EGYLLPLLLI	100
JN0605	51	PTATPADTRP	ARGQAVACN	LQMPHPAWS-	-AVFVAYTTEL	LGFLLPVLA	100
B41795	51	PTMIAALRS	NQWGRSS-CT	INWPGESGAW	YTGFLIYTFL	LGFLVPLFI	100
A39297	51	PIVVFESRTAA	NSLGIVA-CN	MLMPEPADRW	LVGFVLYTFL	MGFLLPVGA	100
p3H2-34	101	CECY----	AK	NMSKKSEASK	KKTAQTVLAV	VVVEALCWL	150
JN0605	101	GLQYLLHVGK	MRAVALRAGW	QQRRSSE---	KKITRLVLMV	VVVEVLCWMP	150
B41795	101	CLQYLFETIK	VKSSGIRVGS	SKRKKSE---	KKVTRMVSIV	VAVFLFCWLP	150
A39297	101	CLCYVLLITAK	MRMVALKAGW	QQRKRSE---	RKITMVMVMV	VMVEVICWMP	150
p3H2-34	151	FY.....					200
JN0605	151	FY.....					200
B41795	151	FY.....					200
A39297	151	FY.....					200

FIGURE 43

5'	10	19	28	37	46	55											
GTG	GGC	ATG	GTG	GGC	AAC	GTC	CTC	GTG	TTC	GGC	TTC	TCC	ATC	AAG			
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---			
Val	Gly	Met	Val	Gly	Asn	Val	Leu	Val	Leu	Trp	Phe	Gly	Ser	Ile	Lys		
64	73	82	91	100	109												
AGG	ACC	CCC	TTC	TCC	GTC	TAC	TTC	CTG	CAC	CTG	GCC	AGC	GCC	GCC	TAC		
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		
Arg	Thr	Pro	Phe	Ser	Val	Tyr	Phe	Leu	His	Leu	Ala	Ser	Ala	Asp	Gly	Ala	Tyr
118	127	136	145	154	163												
CTC	TTC	AGC	AAG	GCC	GTG	TTC	TCC	CTG	CTG	AAC	GCC	GCC	GGC	TTC	CTG	GCC	ACC
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Leu	Phe	Ser	Lys	Ala	Val	Phe	Ser	Leu	Leu	Asn	Ala	Gly	Gly	Phe	Leu	Gly	Thr
172	181	190	199	208	217												
TTC	GCC	CAC	TAT	GTG	CGC	AGC	GTG	GCC	CGG	GTG	CTG	GGG	CTC	TGC	GCC	TTC	GTG
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Phe	Ala	His	Tyr	Val	Arg	Ser	Val	Ala	Arg	Val	Leu	Gly	Leu	Cys	Ala	Phe	Val
226	235	244	253	262													
GCG	GCC	GTG	AGC	CTC	CTG	CCG	GCC	GTG	AGC	ATG	GAG	CGC	TGC	GCG	TCT	G	3'
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Ala	Gly	Val	Ser	Leu	Leu	Pro	Ala	Val	Ser	Met	Glu	Arg	Cys	Ala	Ser		

Year	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099	2100
1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099	2100	

FIGURE 44

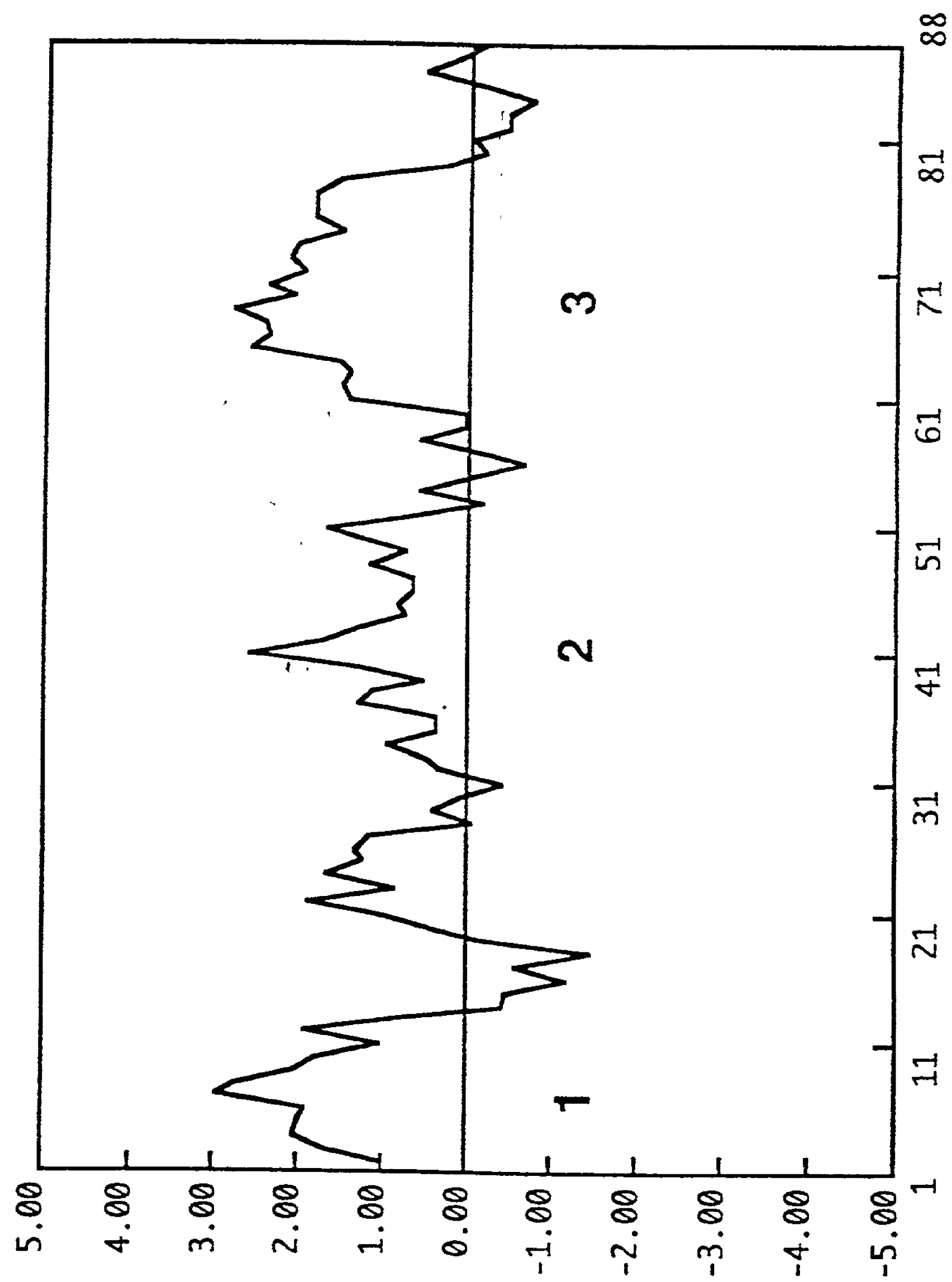


FIGURE 46

1	CAAAGCAACAGGTGCAACCTCAAGGCACTGAAAGCAAGGGGACCCAGCTCACAAGGGCCAAGGGATTGAACC	72
1		1
73	CATAACCGCTCAGAAGATTCTCCGCTGCGGAGAGCTGCGGAGGAGTCCCACCCGTCCAGCTTGCTGACTGC	144
1		1
145	GAGCAGTGAGAGTCGCCTAGACCCGTACCTCTGTGTTCTGGAGCCTGCCGCCCCCGCACGGGAAAGGCTTAG	216
1		1
217	CTCGGGACTTGCAGCACCGCCTCTCTTTAGCCAGGCCAGGCACGAGGATAGTGTGATCGGGCACAGCCAGG	288
1		1
289	GTCGCTCTTCCAGGCTTTCTTGCGGGTTGCGGGAGGTACTAGTTGGAGACGCGCGCGCTCGCTCTCGCCGCT	360
1		1
361	CTGTCTCGGGCCACTCCGTGATCCTAGGCTACCTCCAGAGCCAGTTTTCCCTGGCTGGCACAACCTCTCCAGG	432
1		1
433	GCGCTCCGGTCCGTTGCACAGCGCCCCAAGGGGGTATCCCAAGTAAGTGGAAGTGGCTATGGTGAACCTC	504
1	MetGluLeuAlaMetValAsnLeu	8
505	AGTGAAGGGAATGGGAGCGACCCAGAGCCGCCAGCCCCGAGTCCAGGCCGCTCTTCGGCATTGGCGTGGAG	576
8	SerGluGlyAsnGlySerAspProGluProProAlaProGluSerArgProLeuPheGlyIleGlyValGlu	32
577	AAC TTCATTACGCTGGTAGTGTGTTGGCCTGATTTTCGCGATGGGCGTGCTGGGCAACAGCCTGGTGATCACC	648
32	AsnPheIleThrLeuValValPheGlyLeuIlePheAlaMetGlyValLeuGlyAsnSerLeuValIleThr	56
649	GTGCTGGCGCGCAGCAAACCAGGCAAGCCGCGCAGCACCACCAACCTGTTTATCCTCAATCTGAGCATCGCA	720
56	ValLeuAlaArgSerLysProGlyLysProArgSerThrThrAsnLeuPheIleLeuAsnLeuSerIleAla	80
721	GACCTGGCCTACCTGCTCTTCTGCATCCCTTTTCAGGCCACCGTGTATGCACTGCCACCTGGGTGCTGGGC	792
80	AspLeuAlaTyrLeuLeuPheCysIleProPheGlnAlaThrValTyrAlaLeuProThrTrpValLeuGly	104
793	GCCTTCATCTGCAAGTTTATACACTACTTCTTCACCGTGTCCATGCTGGTGAGCATCTTCACCCCTGGCCGCG	864
104	AlaPheIleCysLysPheIleHisTyrPhePheThrValSerMetLeuValSerIlePheThrLeuAlaAla	128
865	ATGCTGTGTGGATCGCTACGTGGCCATTGTGCACTCGCGGCGCTCCTCCTCCCTCAGGGTGTCCCGCAACGCA	936
128	MetSerValAspArgTyrValAlaIleValHisSerArgArgSerSerSerLeuArgValSerArgAsnAla	152
937	CTGCTGGGCGTGGGCTTCATCTGGGCGCTGTCCATCGCCATGGCCTCGCCGGTGGCCTACCACCAGCGTCTT	1008
152	LeuLeuGlyValGlyPheIleTrpAlaLeuSerIleAlaMetAlaSerProValAlaTyrHisGlnArgLeu	176
1009	TTCCATCGGGACAGCAACCAGACCTTCTGCTGGGAGCAGTGGCCCAACAAGCTCCACAAGAAGGCTTACGTG	1080
176	PheHisArgAspSerAsnGlnThrPheCysTrpGluGlnTrpProAsnLysLeuHisLysLysAlaTyrVal	200
1081	GTGTGCACTTTTCGTCTTTGGGTACCTTCTGCCCTTACTGCTCATCTGCTTTTGCTATGCCAAGGTCCTTAAT	1152
200	ValCysThrPheValPheGlyTyrLeuLeuProLeuLeuLeuIleCysPheCysTyrAlaLysValLeuAsn	224
1153	CATCTGCATAAAAAGCTGAAAAACATGTCAAAAAGTCTGAAGCATCCAAGAAAAAGACTGCACAGACCGTC	1224
224	HisLeuHisLysLysLeuLysAsnMetSerLysLysSerGluAlaSerLysLysLysThrAlaGlnThrVal	248
1225	CTGGTGGTCGTTGTAGTATTTGGCATATCCTGGCTGCCCCATCATGTCTGCCACCTCTGGGCTGAGTTTGA	1296
248	LeuValValValValValPheGlyIleSerTrpLeuProHisHisValValHisLeuTrpAlaGluPheGly	272
1297	GCCTTCCCCTGACGCCAGCTTCCTTCTTCTTCAGAATCACCGCCCATTCCTGGCATAACAGCAACTCCTCA	1368
272	AlaPheProLeuThrProAlaSerPhePhePheArgIleThrAlaHisCysLeuAlaTyrSerAsnSerSer	296
1369	GTGAACCCCATCATATATGCCCTTTCTCTCAGAAAACCTCCGGAAGGCGTACAAGCAAGTGTTCAGTGTCAT	1440
296	ValAsnProIleIleTyrAlaPheLeuSerGluAsnPheArgLysAlaTyrLysGlnValPheLysCysHis	320
1441	GTTTGGGATGAATCTCCACGCAGTGAACTAAGGAAACAAGAGCCGGATGGACACCCCGCCATCCACCAAC	1512
320	ValCysAspGluSerProArgSerGluThrLysGluAsnLysSerArgMetAspThrProProSerThrAsn	344
1513	TGCACCCACGTGTGAAGTTTGGGGAGCCTCCCGACTTCCAGCTCCCATGTGTGTTAGAGAGAGGAGGGCG	1584
344	CysThrHisVal***	349
1585	GAGCGAATTATCAAGTAACATGG	1607
349		349

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FIGURE 47

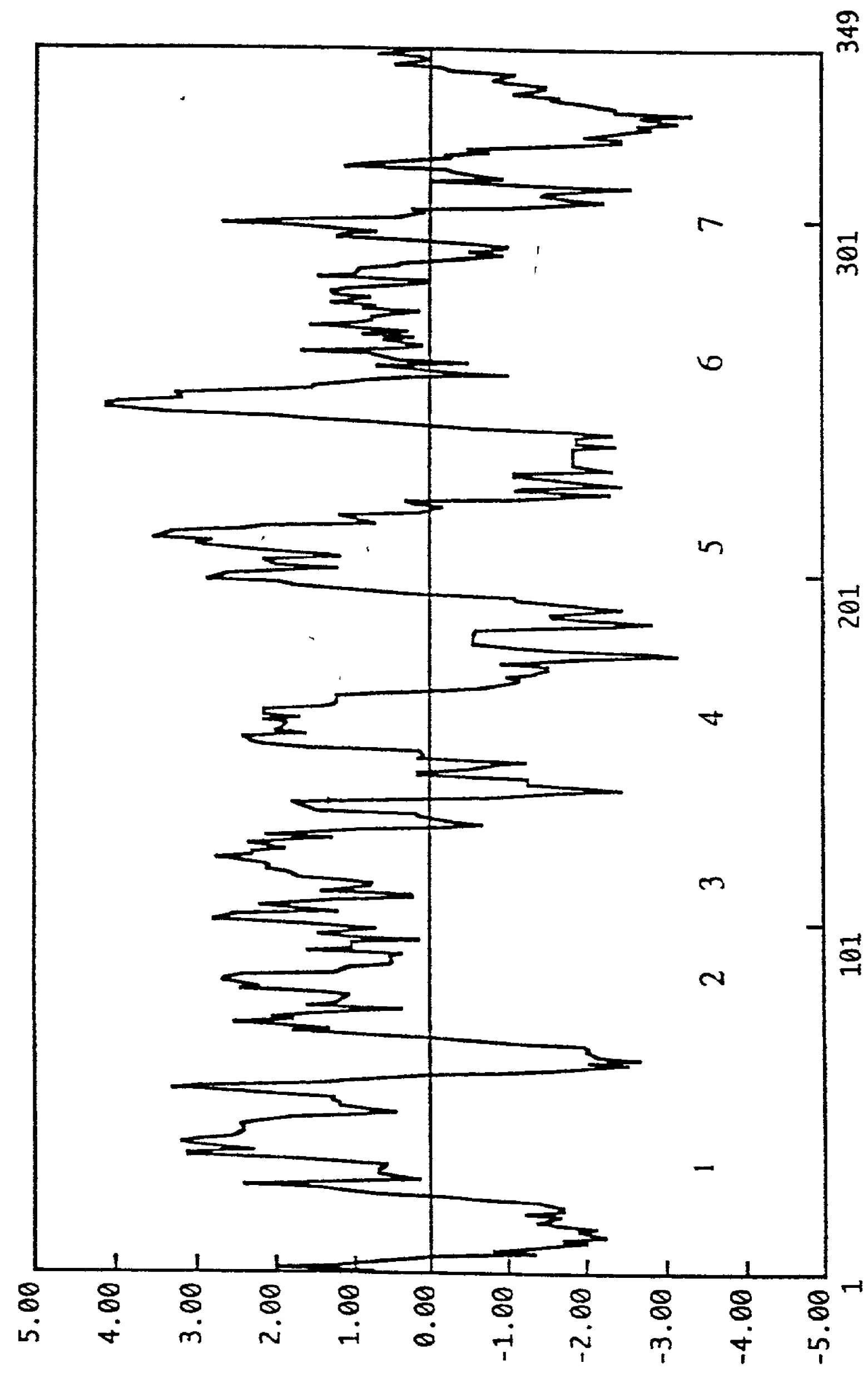


FIGURE 48

MOUSEGALRECE HUMGALAMI	1 1	10 10	20 20	30 30	40 40	50 50
		MELAVNLSE MELAVNLSE	GNVSPERPPA GNVSPERPPA	PEGRPLEGIG PEGRPLEGIG	VENFITLAVF VENFITLAVF	GLIFAGVLG GLIFAGVLG
MOUSEGALRECE HUMGALAMI	51 51	60 60	70 70	80 80	90 90	100 100
		NSLVITVLEAR NSLVITVLEAR	SKPGKPRSTT SKPGKPRSTT	NLEITLNLSTA NLEITLNLSTA	DHAYLLEFCIP DHAAYLLEFCIP	EQATVYALPT EQATVYALPT
MOUSEGALRECE HUMGALAMI	101 101	110 110	120 120	130 130	140 140	150 150
		WVLGAFICKF WVLGAFICKF	THYFTTVSML THYFTTVSML	VSIFTLAAMS VSIFTLAAMS	VPRVYATVHS VDRYVALVHS	RRSSSLRVSR RRSSSLRVSR
MOUSEGALRECE HUMGALAMI	151 151	160 160	170 170	180 180	190 190	200 200
		NALLGVGTLW NALLGVGTLW	ALSTAMASPV ALSTAMASPV	AYHORLEH-R AYHOBLEHPR	DSNOTFCWEO A SNOTFCWEO	WPNKLHKKAY WEDPRHKKAY
MOUSEGALRECE HUMGALAMI	201 201	210 210	220 220	230 230	240 240	250 250
		WVCTFVEGYL WVCTFVEGYL	LPLLLICEFY LPLLLICEFY	AKVLNHLHKK AKVLNHLHKK	LKNMSKKSEA LKNMSKKSEA	SKKKTAQIVL SKKKTAQIVL
MOUSEGALRECE HUMGALAMI	251 251	260 260	270 270	280 280	290 290	300 300
		WVAVFGLISW WVAVFGLISW	LPHHVIHLMA LPHHVIHLMA	EEGAEPITPA EEGVFPLTPA	SEFERITAHG SEFERITAHG	LAYSNSSSVNP LAYSNSSSVNP
MOUSEGALRECE HUMGALAMI	301 301	310 310	320 320	330 330	340 340	350 350
		TIYAFLESENF TIYAFLESENF	RKAYKQVFKC RKAYKQVFKC	HVCDESPRSE HIRKDSILSD	TKENKSRMDT TKENKSRIDT	PPSTINCITHVX PPSTINCITHVX
MOUSEGALRECE HUMGALAMI	351 351	360 360	370 370	380 380	390 390	400 400
	 X.....

FIGURE 49

5'	CTC	GCG	GCT	CTG	GGT	ATG	GAT	CGG	TAT	CTT	CTC	ACC	CTT	CAC	CCA	GTG	TGG	TCC
										Leu	Leu	Thr	Leu	His	Pro	Val	Trp	Ser
	CAA	AAG	CAC	CGA	ACC	TCA	CAC	TGG	GCT	TCC	AGA	GTC	GTT	CTG	GGA	GTC	TGG	CTC
	Gln	Lys	His	Arg	Thr	Ser	His	Trp	Ala	Ser	Arg	Val	Val	Leu	Gly	Val	Trp	Leu
	TCT	GCC	ACT	CCC	TTC	AGC	GTG	CCC	TAT	TTG	GTT	TTC	AGG	GAG	ACA	TAT	GAT	GAC
	Ser	Ala	Thr	Ala	Phe	Ser	Val	Pro	Tyr	Leu	Val	Phe	Arg	Glu	Thr	Tyr	Asp	Asp
	CGT	AAA	GGA	AGA	GTG	ACC	TGC	AGA	AAT	AAC	TAC	GCT	GTG	TCC	ACT	GAC	TGG	GAA
	Arg	Lys	Gly	Arg	Val	Thr	Cys	Arg	Asn	Asn	Tyr	Ala	Val	Ser	Thr	Asp	Trp	Glu
	AGC	AAA	GAG	ATG	CAA	ACA	GTA	AGA	CAA	TGG	ATT	CAT	GCC	ACC	TGT	TTC	ATC	AGC
	Ser	Lys	Glu	Met	Gln	Thr	Val	Arg	Gln	Trp	Ile	His	Ala	Thr	Cys	Phe	Ile	Ser
	CGC	TTC	ATA	CTG	GGC	TTC	CTT	CTG	CCT	TTC	TTA	GTC	ATT	GGC	TTT	TGT	TAT	GAA
	Arg	Phe	Ile	Leu	Gly	Phe	Leu	Leu	Pro	Phe	Leu	Val	Ile	Gly	Phe	Cys	Tyr	Glu
	AGA	GTA	GCC	CGC	AAG	ATG	AAA	GAG	AGG	GGC	CTC	TTT	AAA	TCC	AGC	AAA	CCC	TTC
	Arg	Val	Ala	Arg	Lys	Met	Lys	Glu	Arg	Gly	Leu	Phe	Lys	Ser	Ser	Lys	Pro	Phe
	AAA	GTC	ACG	ATG	ACT	GCT	GTT	ATC	TCT	TTT	TTC	TGT	CCT	GGC	TTC	CCT	ACC	ACA
	Lys	Val	Thr	Met	Thr	Ala	Val	Ile										

TG 3'

FIGURE 50

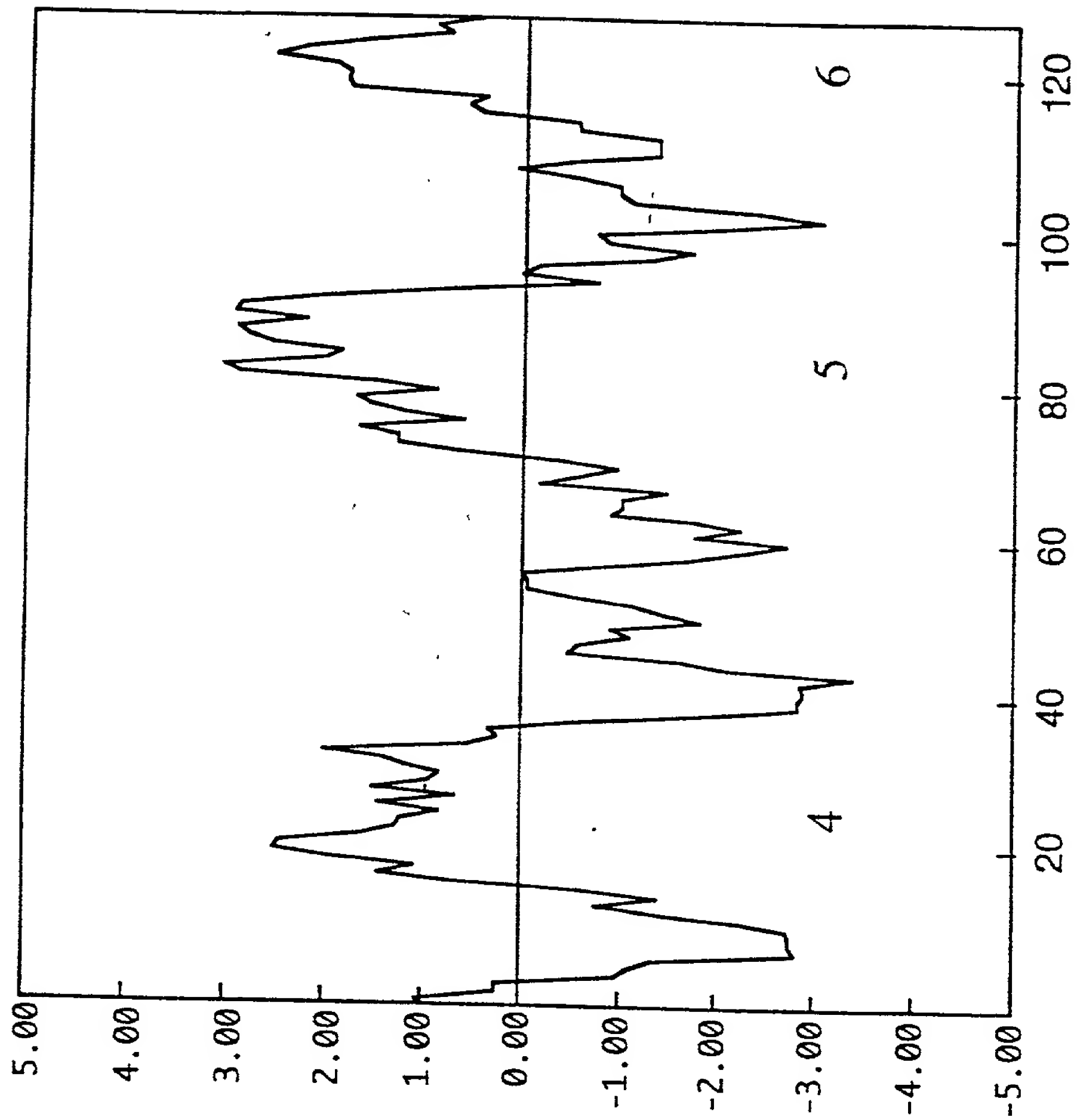


FIGURE 51

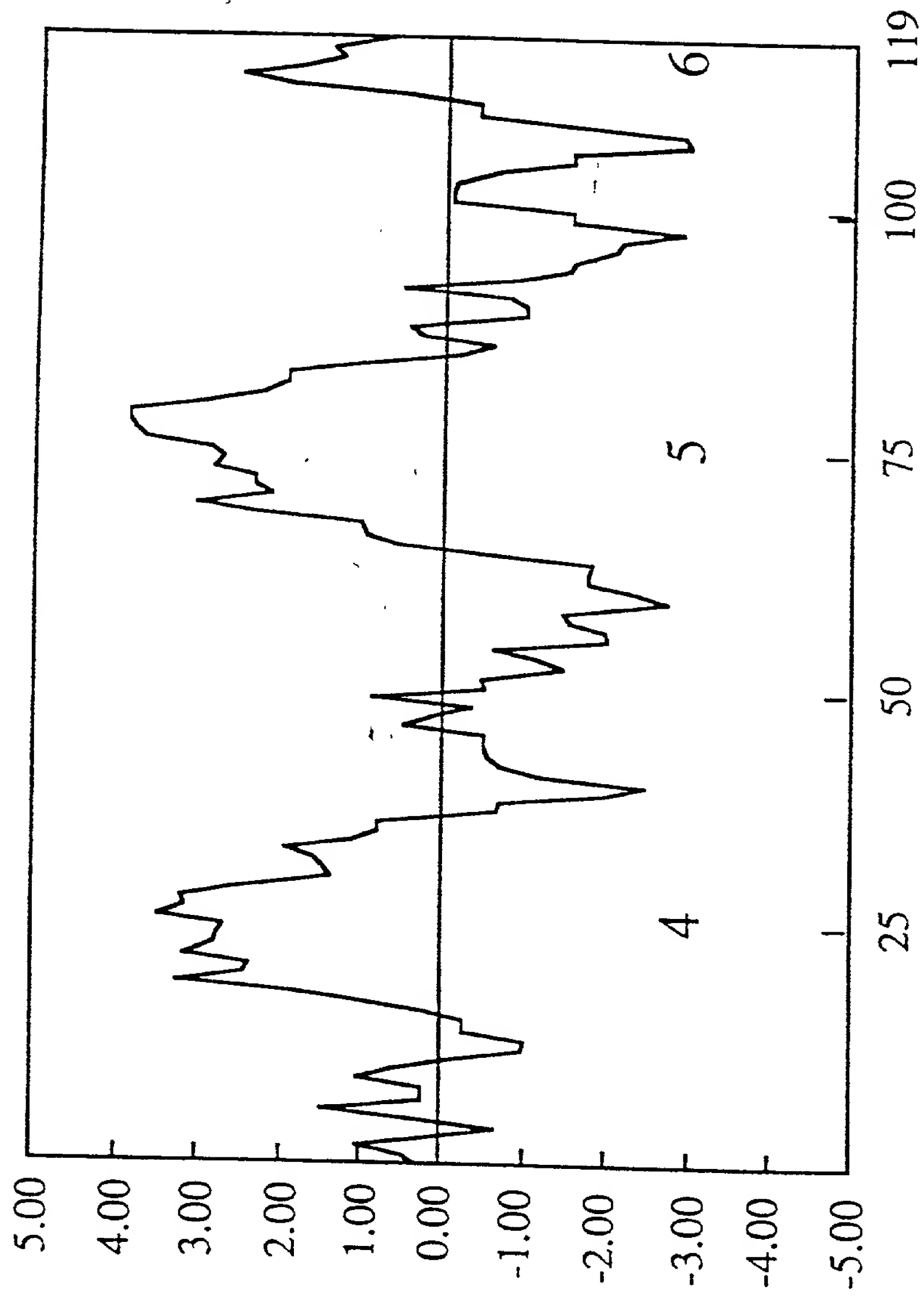
pMJ10	1	10	20	30	40	50	50
B42009	1	1	1	1	1	1	50
JC2014	1	1	1	1	1	1	50
A46520	1	1	1	1	1	1	50
A46525	1	1	1	1	1	1	50
S28787	1	1	1	1	1	1	50
pMJ10	51	60	70	80	90	100	100
B42009	51	51	51	51	51	51	100
JC2014	51	51	51	51	51	51	100
A46520	51	51	51	51	51	51	100
A46525	51	51	51	51	51	51	100
S28787	51	51	51	51	51	51	100
pMJ10	101	110	120	130	140	150	150
B42009	101	101	101	101	101	101	150
JC2014	101	101	101	101	101	101	150
A46520	101	101	101	101	101	101	150
A46525	101	101	101	101	101	101	150
S28787	101	101	101	101	101	101	150

	9			18			27			36			45			54		
5'	CTG	ACT	GCT	CTG	GGG	ACT	GAC	CGG	TAT	TTC	AAG	ATT	GTG	AAG	CCC	CTT	TCC	ACG
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
										Phe	Lys	Ile	Val	Lys	Pro	Leu	Ser	Thr
	63			72			81			90			99			108		
	TCC	TTC	ATC	CAG	TCT	GTG	AAC	TAC	AGC	AAA	CTC	GTC	TCG	CTG	GTG	GTC	TGG	TTG
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	Ser	Phe	Ile	Gln	Ser	Val	Asn	Tyr	Ser	Lys	Leu	Val	Ser	Leu	Val	Val	Trp	Leu
	117			126			135			144			153			162		
	CTC	ATG	CTC	CTC	CTC	GCC	GTC	CCC	AAC	GTC	ATT	CTC	ACC	AAC	CAG	AGA	GTT	AAG
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	Leu	Met	Leu	Leu	Leu	Ala	Val	Pro	Asn	Val	Ile	Leu	Thr	Asn	Gln	Arg	Val	Lys
	171			180			189			198			207			216		
	GAC	GTG	ACG	CAG	ATA	AAA	TGC	ATG	GAA	CTT	AAA	AAC	GAA	CTG	GGC	CGC	CAG	TGG
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	Asp	Val	Thr	Gln	Ile	Lys	Cys	Met	Glu	Leu	Lys	Asn	Glu	Leu	Gly	Arg	Gln	Trp
	225			234			243			252			261			270		
	CAC	AAG	GCG	TCA	AAC	TAC	ATC	TTT	GTG	GGC	ATT	TTC	TGG	CTT	GTG	TTC	CTT	TTG
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	His	Lys	Ala	Ser	Asn	Tyr	Ile	Phe	Val	Gly	Ile	Phe	Trp	Leu	Val	Phe	Leu	Leu
	279			288			297			306			315			324		
	CTA	ATC	ATT	TTC	TAC	ACT	GCT	ATC	ACC	AGG	AAA	ATC	TTT	AAG	TCC	CAC	CTG	AAA
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	Leu	Ile	Ile	Phe	Tyr	Thr	Ala	Ile	Thr	Arg	Lys	Ile	Phe	Lys	Ser	His	Leu	Lys
	333			342			351			360			369			378		
	TCC	AGA	AAG	AAT	TCC	ATC	TCG	GTC	AAA	AAG	AAA	TCT	AGC	CGC	AAC	ATC	TTC	AGC
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	Ser	Arg	Lys	Asn	Ser	Ile	Ser	Val	Lys	Lys	Lys	Ser	Ser	Arg	Asn	Ile	Phe	Ser
	387			396			405			414								
	ATC	GTG	TTT	ATC	CTC	TGT	TGG	CCC	CCC	TAC	CAC	ATC	3'					
	---	---	---	---	---	---	---	---	---	---	---	---						
	Ile	Val																

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FIGURE 53



PMH28	10	20	30	40	50	
P35343	1	FK IYKPLSTS	FIQSVNYSKL	VSLVWVLLML	LLAMPNVLLIT	NQRVKDVTOI
A41795	1	LAFVHATST-	LQKRHLVKF	VCIAMWLLSV	IEALPIILIR	INPVKVNLSL
A47457	1	VAVVHPKAA	RYRRPTVAKV	VNLGVWVESL	LVI LPFWFS	RTPAANSDCIV
	1	VAVVHPLRAA	TYRRPSVAKL	INLGVWLASL	LVT DRIATFA	DTRPARGGO-
PMH28	60	70	80	90	100	
P35343	51	KOME-LKNEL	GRQWHKASNY	IFVGI F-WLV	FLLLIIFYTA	IT-RKIFKSH
A41795	51	VQYEDVGNNT	SRL--RVVLR	ILPQTFGEFLV	PILLIMLFQY	FLLRTLEKAH
A47457	51	ACNM-LMPEP	AQRWLVGFV-	LYTFLLMGEFL	PVGATCLCYV	LIIAKMRMVA
	51	AVAC-NLQWP	HPAWSAVFV-	VYTFLLGEFL	PVLAI GLCYL	LIVGKMRAVA
PMH28	110	120	130	140	150	
P35343	101	LKSRKNSI-S	VKKKSSRNIF	S--IV		
A41795	101	MG----QKHR	AMR----VIF	AVVLY		
A47457	101	LKAGWQQRKR	SEKTTLMVM	MVMV		
	101	LKAGWQQRKR	SEKTTTRLVL	MVMV		

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FIGURE 55

5'		9		18		27		36		45		54						
	GCC	ACC	AAC	GTG	TTC	ATC	CTG	TGT	CTG	GTG	GAC	CTG	CTG	GCT	GCC	CTG	ACC	CTC

										Val	Asp	Leu	Leu	Ala	Ala	Leu	Thr	Leu
		63		72		81		90		99		108						
	ATG	CCT	CTG	GCC	ATG	CTC	TCC	AGC	TCC	GCC	CTC	TTT	GAC	CAC	GCC	CTC	TTT	GGG

	Met	Pro	Leu	Ala	Met	Leu	Ser	Ser	Ser	Ala	Leu	Phe	Asp	His	Ala	Leu	Phe	Gly
		117		126		135		144		153		162						
	GAG	GTG	GCC	TGC	CGC	CTC	TAC	TTG	TTC	CTG	AGC	GTC	TGC	TTT	GTC	AGC	CTG	GCC

	Glu	Val	Ala	Cys	Arg	Leu	Tyr	Leu	Phe	Leu	Ser	Val	Cys	Phe	Val	Ser	Leu	Ala
		171		180		189		198		207		216						
	ATC	CTC	TCG	GTG	TCC	GCC	ATC	AAT	GTG	GAG	CGC	TAC	TAT	TAT	GTG	GTC	CAC	CCC

	Ile	Leu	Ser	Val	Ser	Ala	Ile	Asn	Val	Glu	Arg	Tyr	Tyr	Tyr	Val	Val	His	Pro
		225		234		243		252		261		270						
	ATG	CGC	TAT	GAG	GTG	CGC	ATG	AAA	CTG	GGG	CTG	GTG	GCC	TCT	GTG	CTG	GTG	GGC

	Met	Arg	Tyr	Glu	Val	Arg	Met	Lys	Leu	Gly	Leu	Val	Ala	Ser	Val	Leu	Val	Gly
		279		288		297		306		315		324						
	GTG	TGG	GTG	AAG	GCC	CTG	GCC	ATG	GCT	TCT	GTG	CCA	GTG	TTG	GGA	AGG	GTG	TCC

	Val	Trp	Val	Lys	Ala	Leu	Ala	Met	Ala	Ser	Val	Pro	Val	Leu	Gly	Arg	Val	Ser
		333		342		351		360		369		378						
	TGG	GAG	GAA	GGC	CCT	CCC	AGT	GTC	CCC	CCA	GGC	TGT	TCA	CTC	CAA	TGG	AGC	CAC

	Trp	Glu	Glu	Gly	Pro	Pro	Ser	Val	Pro	Pro	Gly	Cys	Ser	Leu	Gln	Trp	Ser	His
		387		396		405		414		423		432						
	AGT	GCC	TAC	TGC	CAG	CTT	TTC	GTG	GTG	GTC	TTC	GCC	GTC	CTC	TAC	TTC	CTG	CTG

	Ser	Ala	Tyr	Cys	Gln	Leu	Phe	Val	Val	Val	Phe	Ala	Val	Leu	Tyr	Phe	Leu	Leu
		441		450		459		468		477		486						
	CCC	CTG	CTC	CTC	ATC	CTT	GTG	GTC	TAC	TGC	AGC	ATG	TTC	CGG	GTG	GCT	CGT	GTG

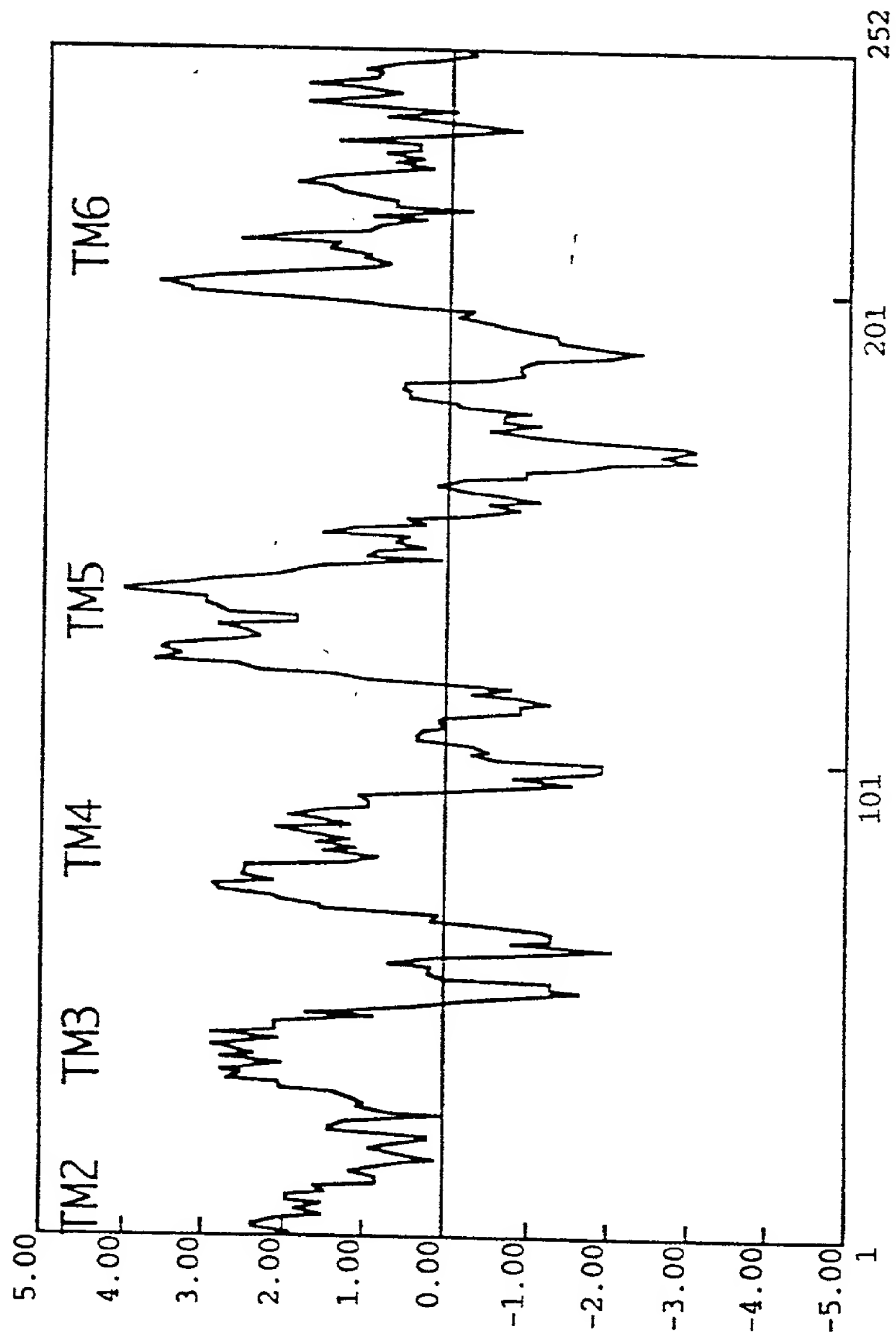
	Pro	Leu	Leu	Leu	Ile	Leu	Val	Val	Tyr	Cys	Ser	Met	Phe	Arg	Val	Ala	Arg	Val
		495		504		513		522		531		540						
	GCT	GCC	ATG	CAG	CAC	GGG	CCG	CTG	CCC	ACG	TGG	ATG	GAG	ACG	CCC	CGG	CAA	CGC

	Ala	Ala	Met	Gln	His	Gly	Pro	Leu	Pro	Thr	Trp	Met	Glu	Thr	Pro	Arg	Gln	Arg

FIGURE 56

549	558	567	576	585	594
TCC GAG TCT CTC AGC	AGC CGC TCC ACT ATG GTC	ACC AGC TCG GGG GCC CCG CAG			
---	---	---	---	---	---
Ser Glu Ser Leu Ser	Ser Arg Ser Thr Met Val Thr Ser Ser Gly Ala Pro Gln				
603	612	621	630	639	648
ACC ACC CCT CAC CGG	ACG TTT GGC GGA GGG AAG GCA GCA GTG	GTC CTC CTG GCT			
---	---	---	---	---	---
Thr Thr Pro His Arg Thr Phe Gly Gly Gly Lys Ala Ala Val Val Leu Leu Ala					
657	666	675	684	693	702
GTG GGA GGA CAG TTC CTG CTC TGT TGG TTG CCC TAC TTC TCC TTC CAC CTC TAT					
---	---	---	---	---	---
Val Gly Gly Gln Phe Leu Leu Cys Trp Leu Pro Tyr Phe Ser Phe His Leu Tyr					
711	720	729	738	747	756
GTG GCC CTG AGC GCT CAG CCC ATT GCA GCG GGG CAG GTG GAG AAC GTG GTG ACC					
---	---	---	---	---	---
Val Ala Leu Ser Ala Gln Pro Ile Ala Ala Gly Gln Val Glu Asn Val Val Thr					
765	774	783	792	801	810
TGG ATT GGC TAC TTC TGC TTC ACC TCC	<u>AAC CCT CTC CTC TAT TCC TTC CTC CCT</u>	3'			
---	---	---	---	---	---
Trp Ile Gly Tyr Phe Cys Phe Thr Ser					

FIGURE 57



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FIGURE 58

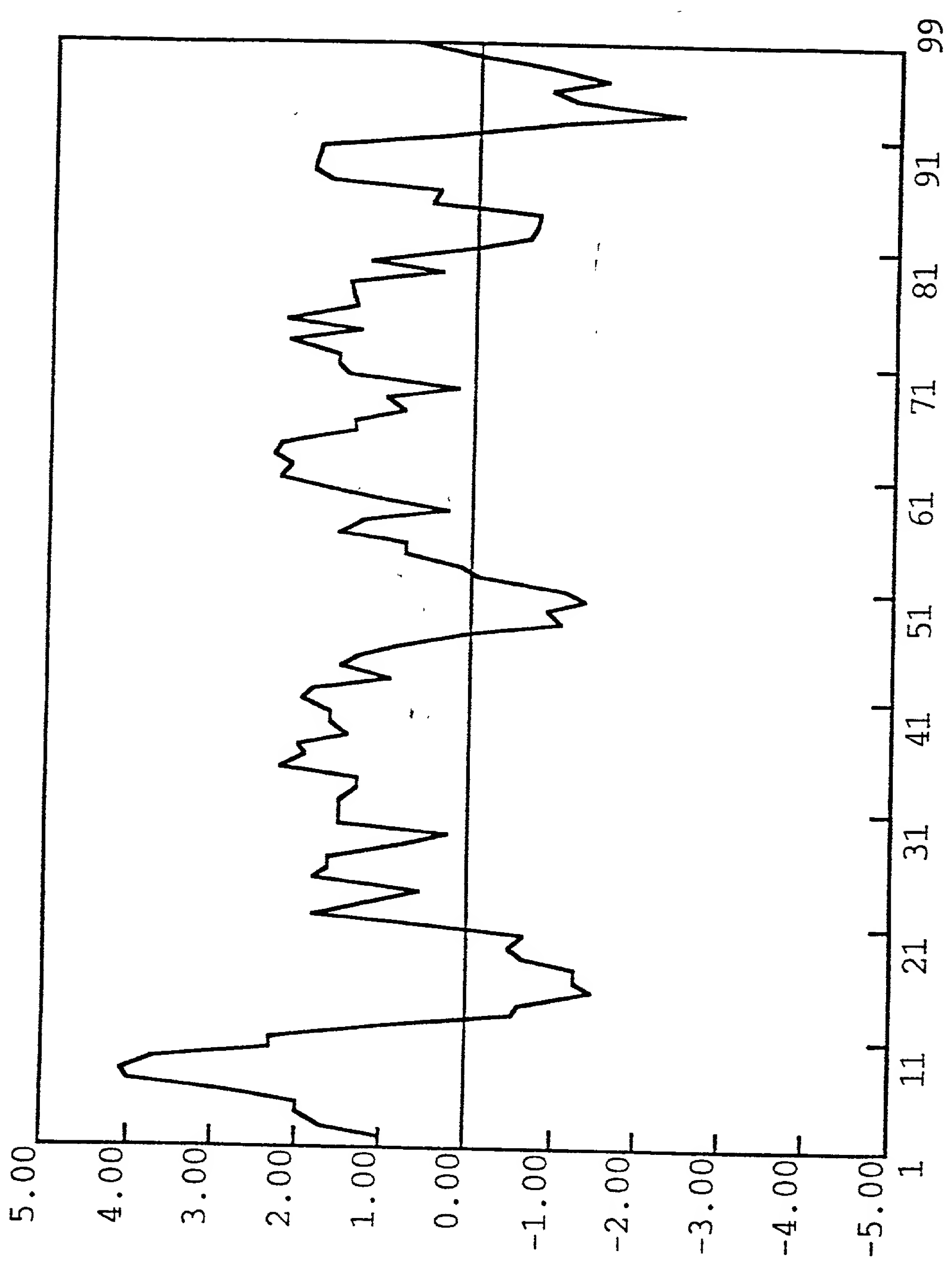
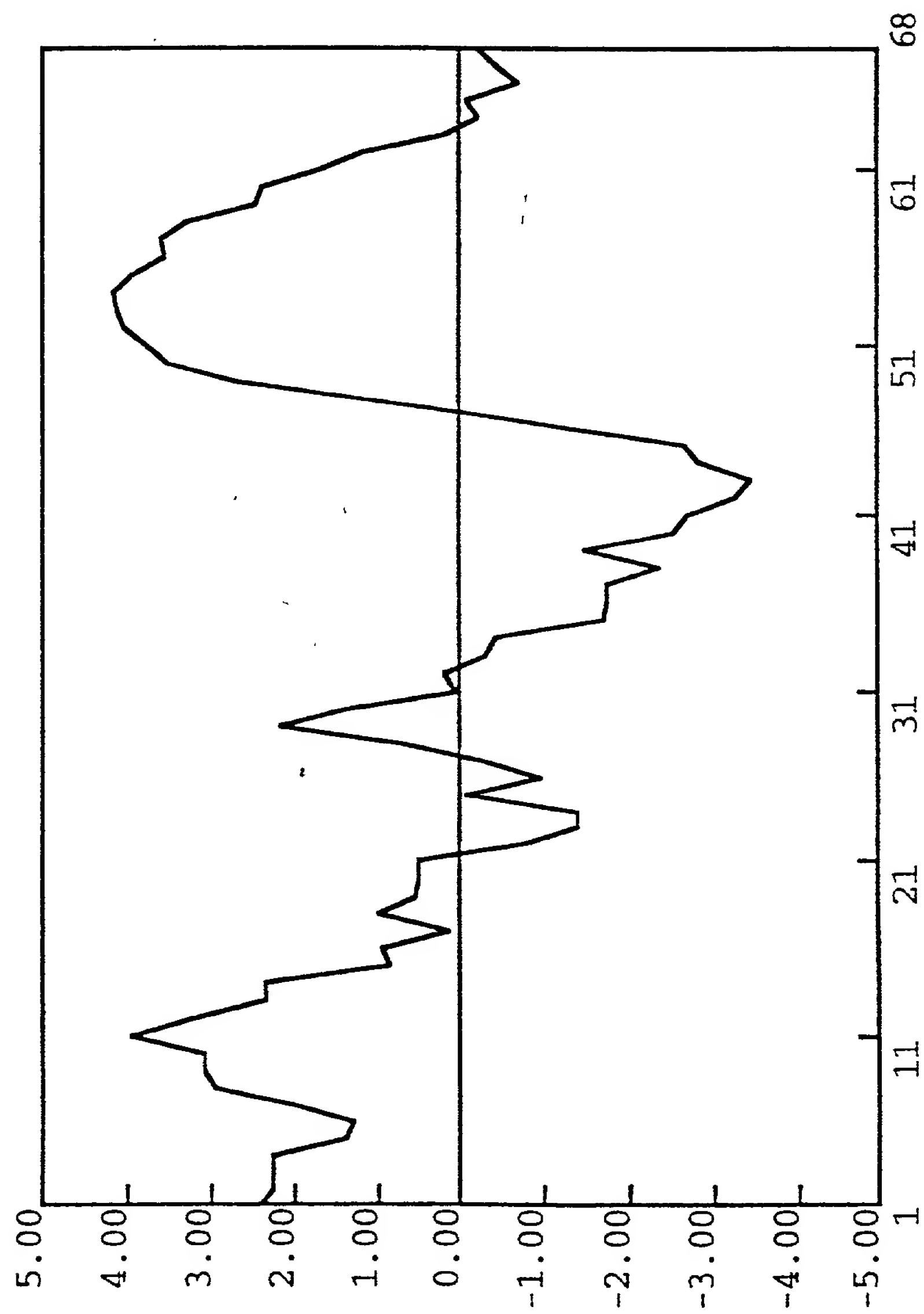


FIGURE 59



at the 100% level of identity. The sequences were aligned using the Clustal W algorithm. The alignment was inspected and corrected where necessary. The sequences were then translated into amino acid sequences. The sequences were then compared using the BLAST algorithm. The results are shown in the table below.

FIGURE 60

p19P2 S12863	10 1 VGMVGNVLV 1 LCVSGLAL	20 LVITARVRLH IIILKQKEMR	30 NVVNFLLCNE NVVNFLLVNL	40 ALSDVLMCTA SFSDLLAVAM	50 CVPPLTLAYAF CLPFTFVYIL
p19P2 S12863	60 51 EPRGWNFCGG 51 MDH-WVFCET	70 LCHLVFLQF MCKLNPVQC	80 VIVVVSVEFL VSITVSIFFSL	90 TTIAVDRLVV VLIAVERHQL	100 LVHPLRRRI- IINPRGWRPN
p19P2 S12863	110 101 NRHAYIGITV	120 IWVLAVALSS	130 PFVIYQILTD	140 EPFQNVSLAA	150 FKDKYVCFDK
p19P2 S12863	160 151 -----GLLV 151 FPSDSHRLSY	170 TYLLPLLVIL TTLVLVQVF	180 LS-----Y GPLCFIFICY	190 VRVSVKLRNR FKIYIRLKR	200 VVPGCCVTQSQ NNMMDKIRDS
p19P2 S12863	210 201 ADWDRARRRR 201 KYRSSETKRI	220 TFCLLVWVVV NVMELSTVVA	230 VFALCWLPYY -FAVCWLPILT	240	250

FIGURE 61

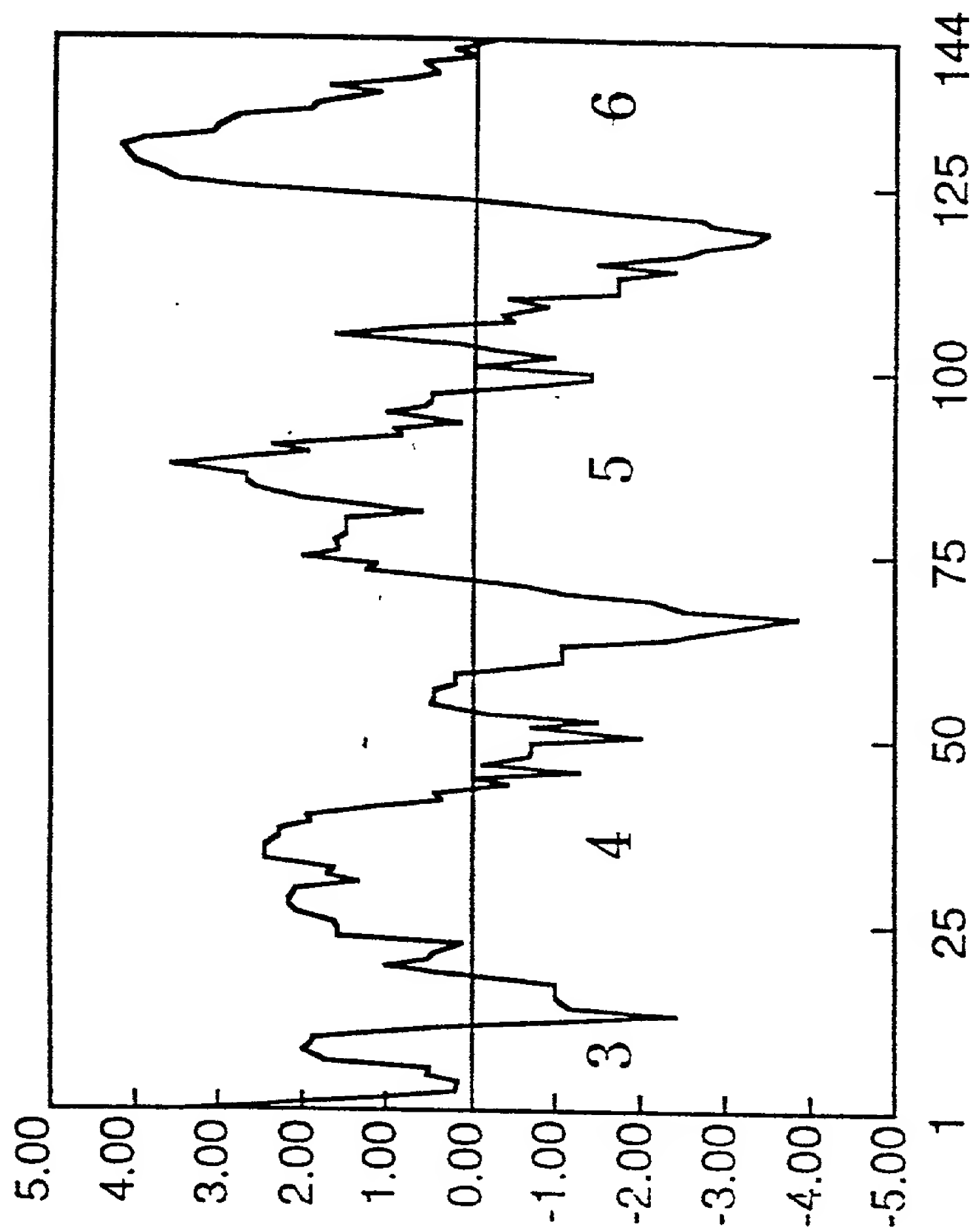
p19P2	1	10	20	30	40	50	
PG3-2/PG1-10	1	VGMVGNVLELV	LVIAVRRLH	NVTNFIIGNL	ALSDVLMCTA	CVPLTLAYAF	50
	1	VGMVGNVLELV	LVIAVRRLY	NVTNFIIGNL	ALSDVLMCTA	CVPLTLAYAF	50
p19P2	51	60	70	80	90	100	
PG3-2/PG1-10	51	EPRGMVFGGG	LCHLVFFLOP	VTVVSVEITL	TTIAVDRIYV	LVHPLRRRI	100
	51	EPRGMVFGGG	LCHLVFFLOA	VTVVSVEITL	TTIAVDRIYV	LVHPLRRRI	100
p19P2	101	110	120	130	140	150	
PG3-2/PG1-10	101	LRLSAYAVLA	IWL SAVLAL	PAAVHTYHVE	LKPHDVRLCE	EFWGSQERQR	150
	151	160	170	180	190	200	
p19P2	151	GLLLV	TYLLPPLLVIL	LSYVRVSVKL	RNRVVEGCVT	QSOADWDRAR	200
PG3-2/PG1-10	151	QLYAWGLLLV	TYLLPPLLVIL	LSYVRVSVKL	RNRVVEGCVT	QSOADWDRAR	200
p19P2	201	210	220	230	240	250	
PG3-2/PG1-10	201	RRRTFCLLLV	VVVFATCWL	PYY.....	250
	201	RRRTFCLLLV	VVVFATCWL	PFF.....	250

FIGURE 62

5'	CTG	TGT	GTC	ATC	GCG	GTG	GAT	AGG	TAC	GTG	GTT	CTG	GTG	CAC	CCG	CTA	CGT	CGG	54
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	Leu	Cys	Val	Ile	Ala	Val	Asp	Arg	Tyr	Val	Val	Leu	Val	His	Pro	Leu	Arg	Arg	
	CGC	ATT	TCA	CTG	AGG	CTC	AGC	GCC	TAC	GCG	GTG	CTG	GCC	ATC	TGG	GCT	CTA	TCT	108
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	Arg	Ile	Ser	Leu	Arg	Leu	Ser	Ala	Tyr	Ala	Val	Leu	Gly	Ile	Trp	Ala	Leu	Ser	
	GCA	GTG	CTG	GCG	CTG	CCG	GCC	GCG	GTG	CAC	ACC	TAC	CAT	GTG	GAG	CTC	AAG	CCC	162
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	Ala	Val	Leu	Ala	Leu	Pro	Ala	Ala	Val	His	Thr	Tyr	His	Val	Glu	Leu	Lys	Pro	
	CAC	GAC	GTG	AGC	CTC	TGC	GAG	GAG	TTC	TGG	GCG	TCG	CAG	GAG	CGC	CAA	CGC	CAG	216
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	His	Asp	Val	Ser	Leu	Cys	Glu	Glu	Phe	Trp	Gly	Ser	Gln	Glu	Arg	Gln	Arg	Gln	
	ATC	TAC	GCC	TGG	GGG	CTG	CTT	CTG	GCG	ACC	TAT	TTC	CTC	CCC	CTG	CTG	GCC	ATC	270
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	Ile	Tyr	Ala	Trp	Gly	Leu	Leu	Leu	Gly	Thr	Tyr	Leu	Leu	Pro	Leu	Leu	Ala	Ile	
	CTC	CTG	TCT	TAC	GTA	CGG	GTG	TCA	GTG	AAG	CTG	AGG	AAC	CGC	GTG	GTG	CCT	GGC	324
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	Leu	Leu	Ser	Tyr	Val	Arg	Val	Ser	Val	Lys	Leu	Arg	Asn	Arg	Val	Val	Pro	Gly	
	AGC	GTG	ACC	CAG	AGT	CAA	GCT	GAC	TGG	GAC	CGA	GCG	CGT	CGC	CGC	CGC	ACT	TTC	378
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	Ser	Val	Thr	Gln	Ser	Gln	Ala	Asp	Trp	Asp	Arg	Ala	Arg	Arg	Arg	Arg	Thr	Phe	
	TGT	CTG	CTG	GTG	GTG	GTG	GTG	GTA	GTG	TTC	ACG	CTC	TGC	TGG	CTG	CCC	TTC	TAC	432
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	Cys	Leu	Leu	Val	Val	Val	Val	Val	Val	Phe	Thr	Leu	Cys	Trp	Leu	Pro	Phe	Tyr	

CT 3'

FIGURE 64



6 5 / 7 9

FIGURE 65

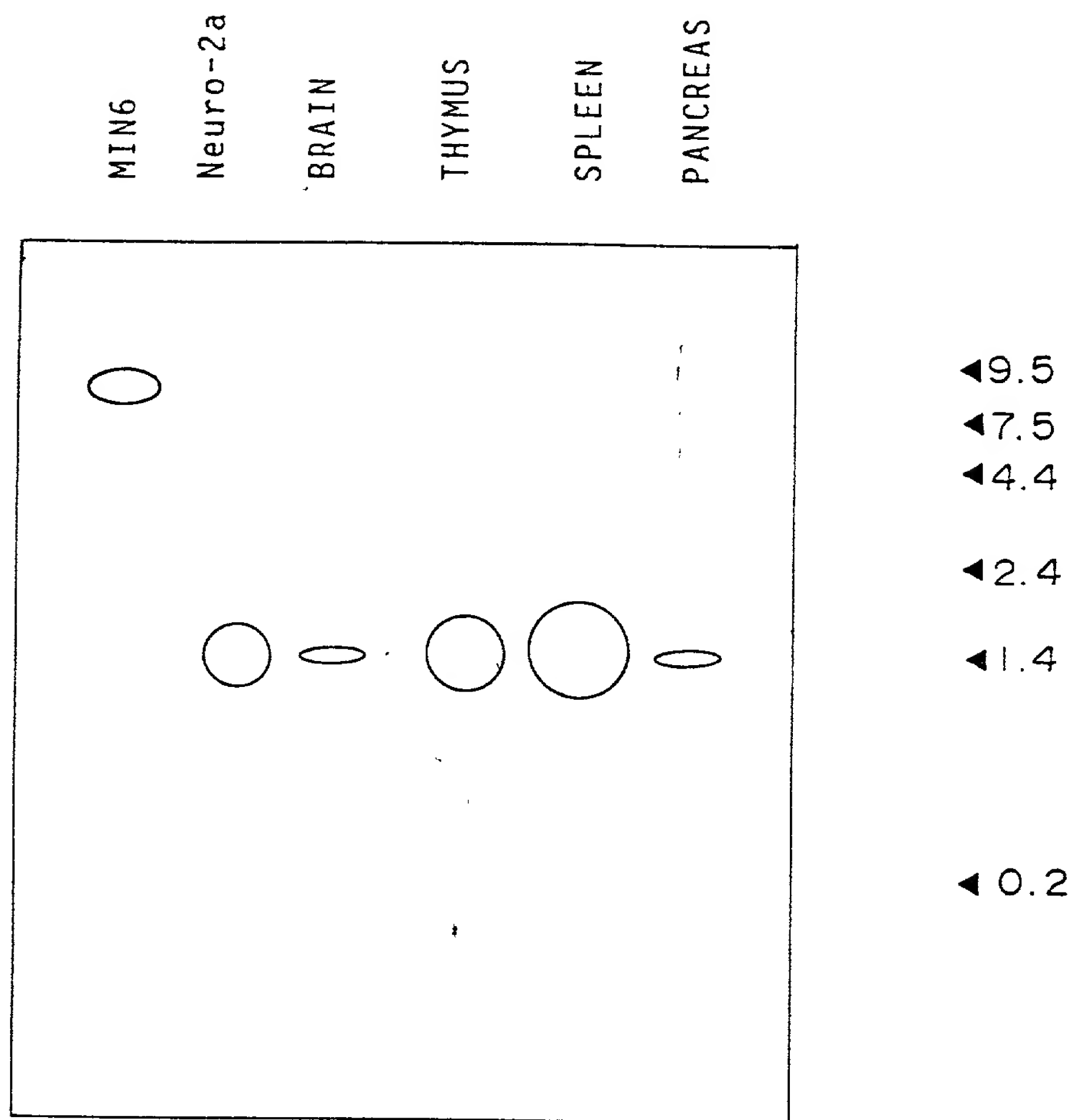
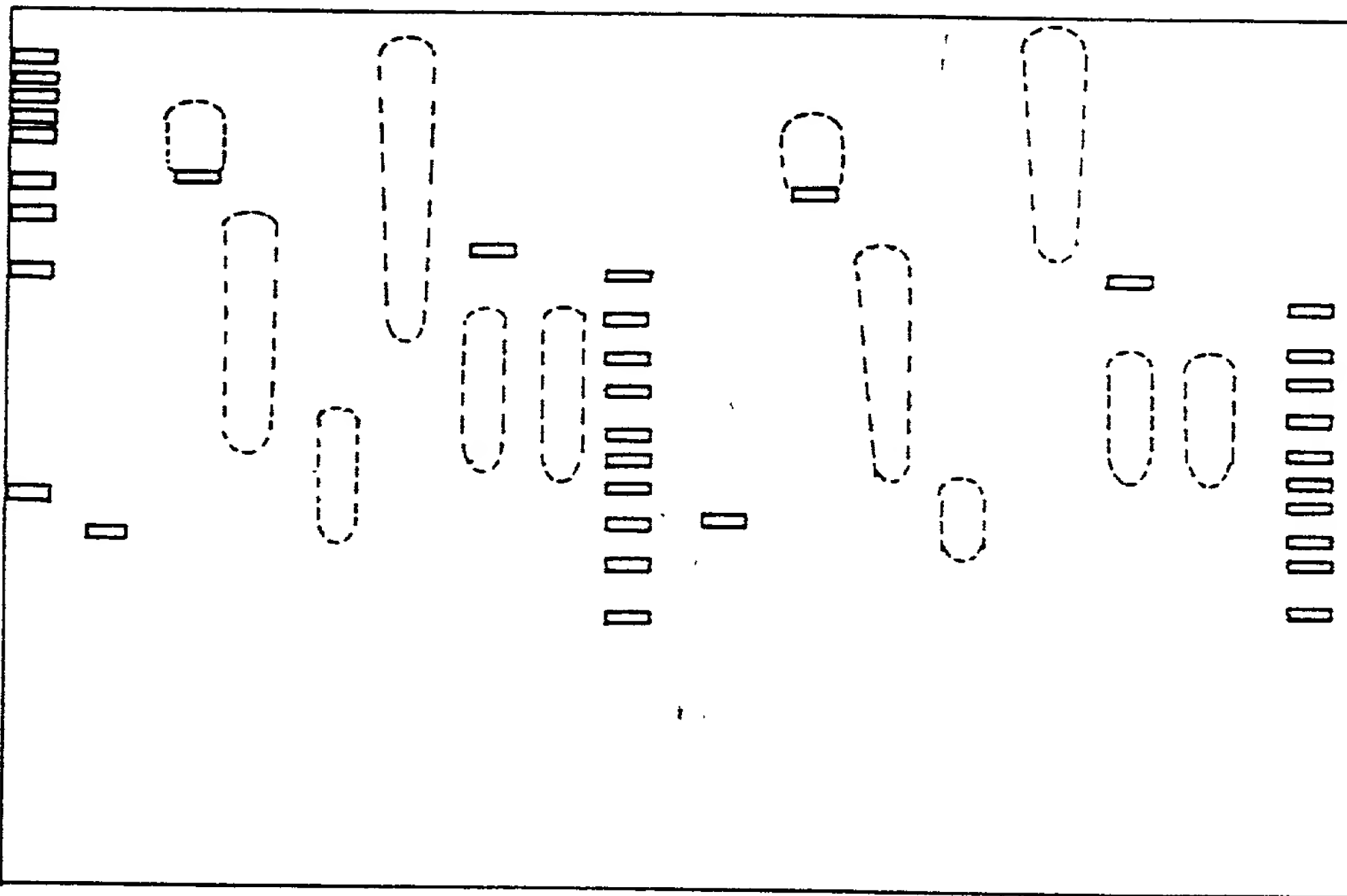


FIGURE 66

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17



6 7 / 7 9

FIGURE 67

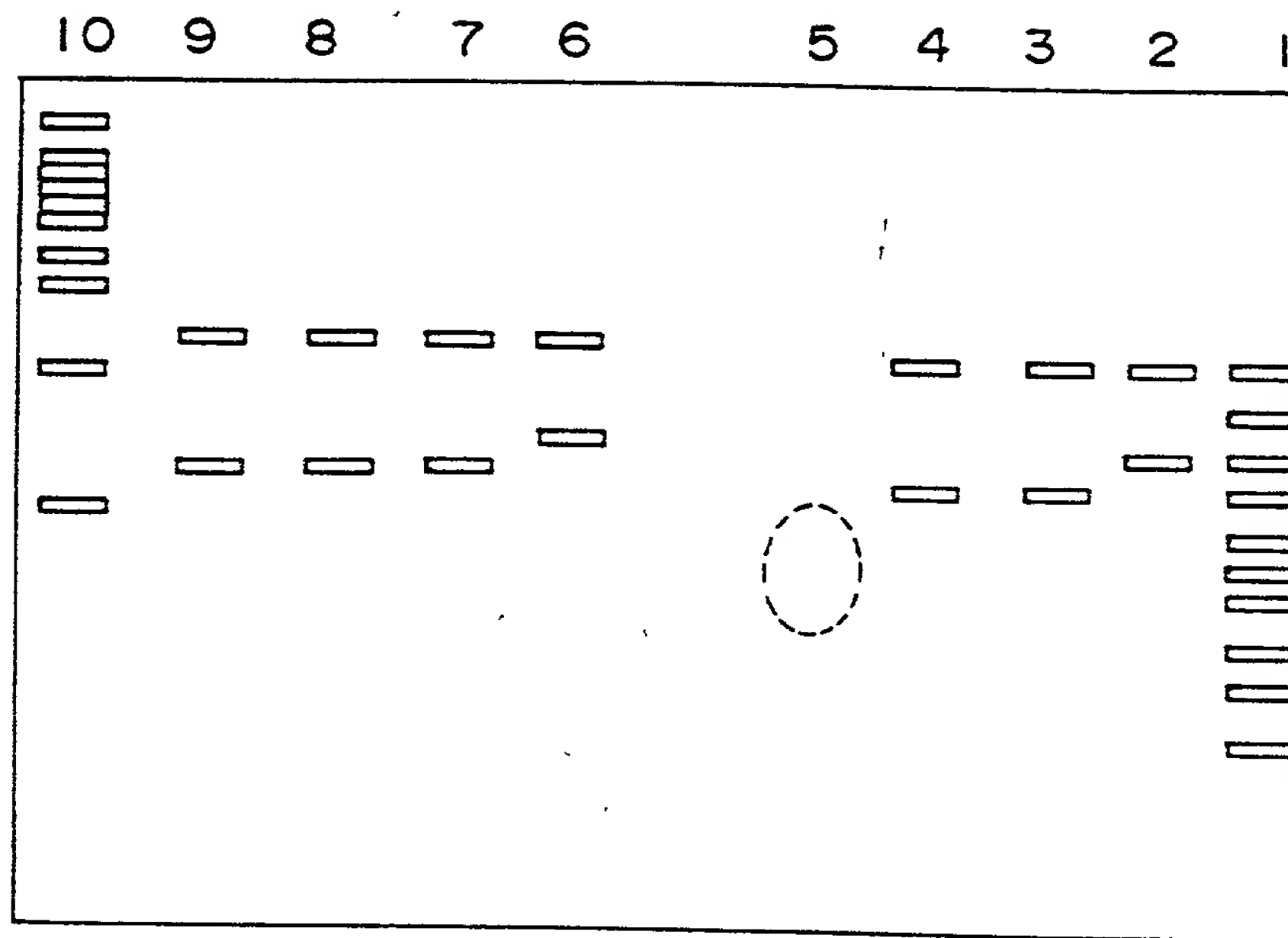


FIGURE 68

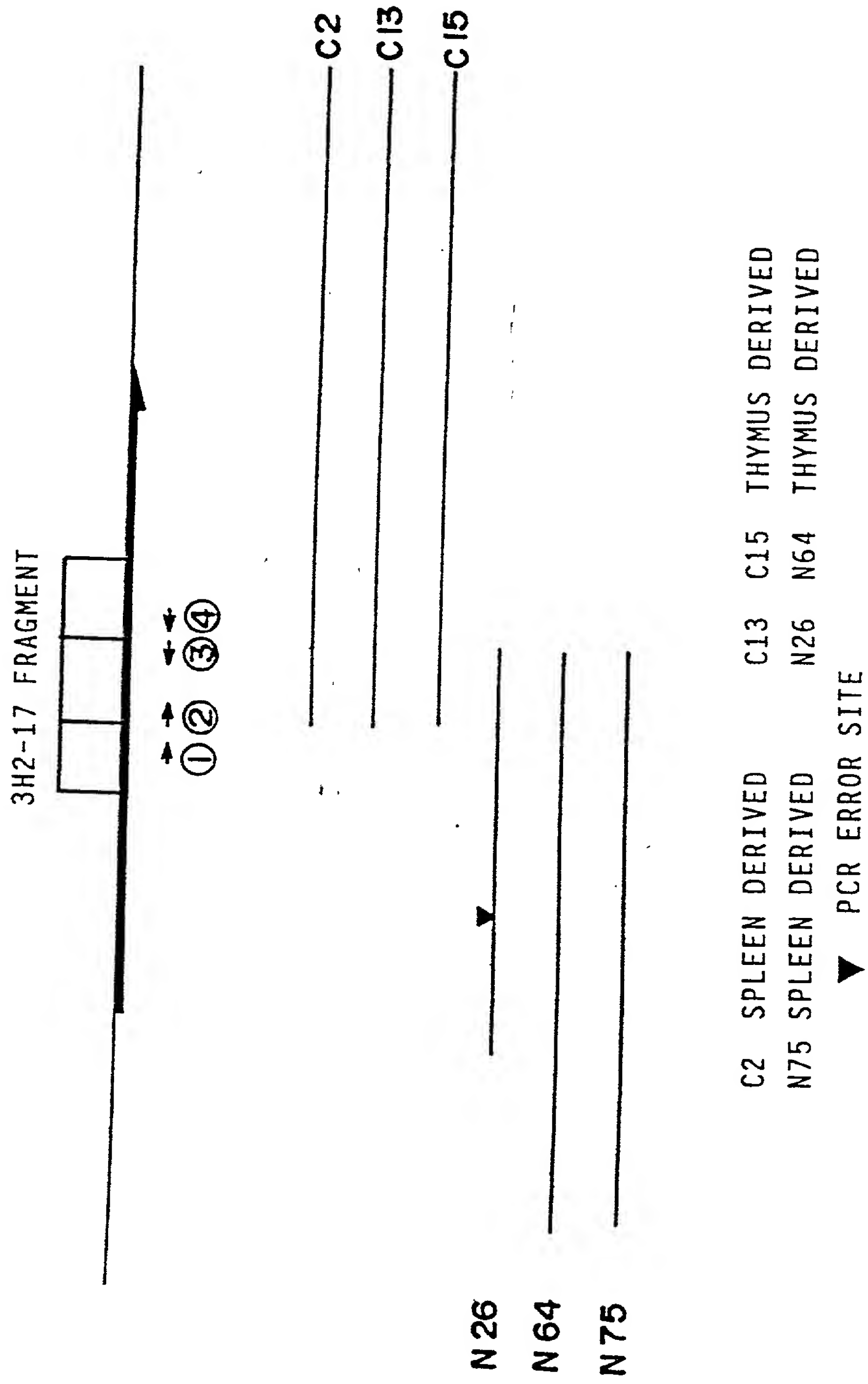


FIGURE 69

1	GAGCATAGGAAAGGCTGACAGGCAGTTATGGAGCAGGACCAATGGCACCATCCAGGCTCCA	60
1	MetGluGlnAspAsnGlyThrIleGlnAlaPro	11
61	GGCTTGCCGCCACCACCTGCGTCTACCGTGAGGATTTCAAGCGACTGCTGCTAACCCCG	120
11	GlyLeuProProThrThrCysValTyrArgGluAspPheLysArgLeuLeuLeuThrPro	31
121	GTATACTCGGTGGTGGTGGTGGCCTGCCACTGAACATCTGCGTCATTGCCCAGATC	180
31	ValTyrSerValValLeuValValGlyLeuProLeuAsnIleCysValIleAlaGlnIle	51
181	TGCGCATCCCGCCGGACCCTGACCCGTTCCGCTGTGTACACCTGAACCTGGCACTGGCG	240
51	CysAlaSerArgArgThrLeuThrArgSerAlaValTyrThrLeuAsnLeuAlaLeuAla	71
241	GACCTGATGTATGCCTGTTCACCTACCCCTACTTATCTATAACTACGCCAGAGGGGACCAC	300
71	AspLeuMetTyrAlaCysSerLeuProLeuLeuIleTyrAsnTyrAlaArgGlyAspHis	91
301	TGGCCCTTCGGAGACCTCGCCTGCGCTTTGTACGCTTCCTCTCTATGCCAATCTACAT	360
91	TrpProPheGlyAspLeuAlaCysArgPheValArgPheLeuPheTyrAlaAsnLeuHis	111
361	GGCAGCATCTGTTCCTCACCTGCCATTAGCTTCCAGCGCTACCTGGGCATCTGCCACCCC	420
111	GlySerIleLeuPheLeuThrCysIleSerPheGlnArgTyrLeuGlyIleCysHisPro	131
421	CTGGCTTCCTGGCACAAGCGTGGAGGTGCGCGTGTGCTTGGGTAGTGTGTGGAGTCGTG	480
131	LeuAlaSerTrpHisLysArgGlyGlyArgArgAlaAlaTrpValValCysGlyValVal	151
481	TGGCTGGCTGTGACAGCCAGTGCCTGCCCACGGCAGTCTTTGCTGCCACAGGCATCCAG	540
151	TrpLeuAlaValThrAlaGlnCysLeuProThrAlaValPheAlaAlaThrGlyIleGln	171
541	CGCAACCGCACTGTGTGCTACGACCTGAGCCACCCATCCTGTCTACTCGCTACCTGCCC	600
171	ArgAsnArgThrValCysTyrAspLeuSerProProIleLeuSerThrArgTyrLeuPro	191
601	TATGGTATGGCCCTCACGGTCATCGGCTTCTTTGCTGCCCTTCATAGCCTTACTGGCTTGT	660
191	TyrGlyMetAlaLeuThrValIleGlyPheLeuLeuProPheIleAlaLeuLeuAlaCys	211
661	TATTGTGCGATGGCCCCCGCGCTGTGTGCGCCAGGATGGCCCAGCAGGTCTGTGGCCCCAA	720
211	TyrCysArgMetAlaArgArgLeuCysArgGlnAspGlyProAlaGlyProValAlaGln	231
721	GAGCGGCGCAGCAAGGCGGCTCGTATGGCTGTGGTGGTGGCAGCTGTCTTTGCCATCAGC	780
231	GluArgArgSerLysAlaAlaArgMetAlaValValValAlaAlaValPheAlaIleSer	251
781	TTCTGCGCTTTCCACATCACCAAGACAGCCTACTTGGCTGTGCGCTCCACGCCCGGTGTC	840
251	PheLeuProPheHisIleThrLysThrAlaTyrLeuAlaValArgSerThrProGlyVal	271
841	TCTTGCCCTGTGCTGGAGACCTTCGCTGCTGCCTACAAAGGCACTCGGGCCCTTCGCCAGT	900
271	SerCysProValLeuGluThrPheAlaAlaAlaTyrLysGlyThrArgProPheAlaSer	291
901	GTCAACAGTGTCTTGGACCCCATCTCTTCTACTTCACACAACAGAAGTTCGGCGGCAA	960
291	ValAsnSerValLeuAspProIleLeuPheTyrPheThrGlnGlnLysPheArgArgGln	311
961	CCCCACGATCTCTTACAGAGGCTCACAGCCAAGTGGCAGAGGCAGAGAGTCTGAGGCCCC	1020
311	ProHisAspLeuLeuGlnArgLeuThrAlaLysTrpGlnArgGlnArgVal***	329

all the data in this report are from the same source and are not to be taken as a representation of the views of the Department of Defense.

FIGURE 70

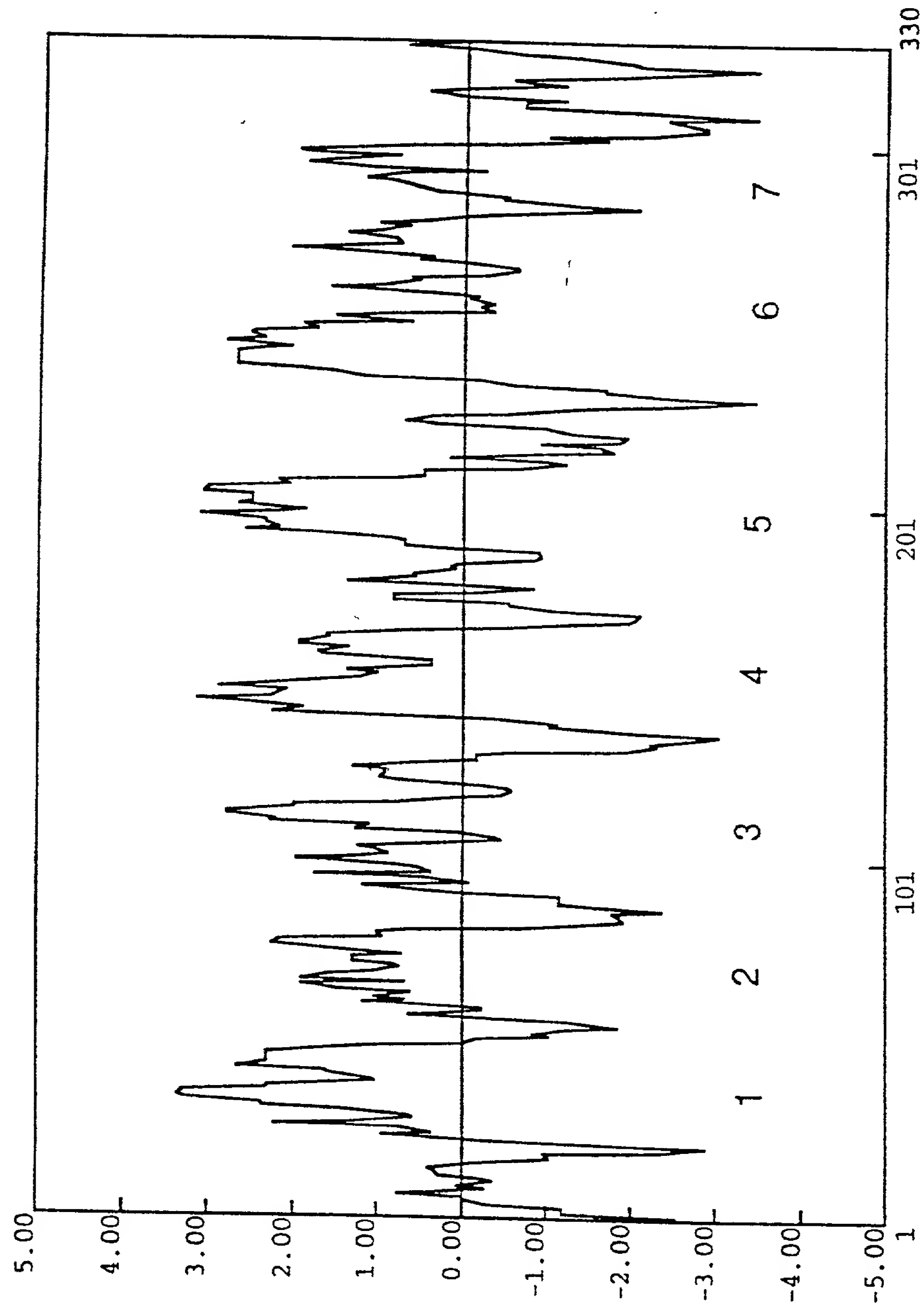


FIGURE 71

75+13, CODING	1	10	20	30	40	50	
P2UR_MOUSE	1	MAADLEFANS	TINGIWEGDE	LGY-----	---KREFN-E	DEKRVLLP-	50
P2YR_CHICK	1	ATEALISAAL	---NGI-3-PE	ELAGGWAAGN	AINKCSLTXT	GEQFYIL-ET	50
75+13, CODING	51	60	70	80	90	100	
P2UR_MOUSE	51	VSVVWVVS	-EPENICVIA	QI--CASRRI	LTR-SAVVIL	NLALADLMA	100
P2YR_CHICK	51	VSYGVVVC	-LCNVVALY	-HSLC-RLKI	ANA-SITYGE	HRAVSESTVA	100
	51	V-YILVFTTG	FLG-NSVALW	M-E-VFHMER	VSGIS-VYME	NLALADFLW	100
75+13, CODING	101	110	120	130	140	150	
P2UR_MOUSE	101	CSLEPLLYNY	ARG-DHWPFQ	ELACRFVRSL	RYANLEPSSTL	ELICTISPORY	150
P2YR_CHICK	101	ASLEPLLYTY	ARG-DHWPFQ	TVLCKLVREL	RYANLEPSSTL	ELICTISVHRC	150
	101	LTLPALIFYY	FNKTP-NEEG	DVMCKLQRET	SHVNLKGSSTL	ELICTISVHRV	150
75+13, CODING	151	160	170	180	190	200	
P2UR_MOUSE	151	LGICHPLASW	HKEGSR-RAA	WVCGVVRILA	VTACCL-ETA	VEAA-IGTOR	200
P2YR_CHICK	151	LGVALPLHSL	--SWGRARYA	RRVAAVWAVL	VLA-CQAEVL	YFVT-LSVRG	200
	151	TGWHPKSL	G-SLKKKN-A	VYVSSLVVAL	VWAVIA-ETE	-FYSGIGVRR	200
75+13, CODING	201	210	220	230	240	250	
P2UR_MOUSE	201	NRI-VCYDLS	PPI-E-SIRY	LPYGNALIVT	GELLDEETALL	ACYCRVARRL	250
P2YR_CHICK	201	TR-LTCHDTS	ARE-LPSHEV	A-YSSVMGL	LEAVHESVIL	VCYVLMARRL	250
	201	NKIMICYDIT	ADEYLSMEV	--YSNCTIVF	MECIHFIIVL	G-CYGLIVKAL	250
75+13, CODING	251	260	270	280	290	300	
P2UR_MOUSE	251	CRODGRA-CP	VAQERSKAA	--RMAVVAA	VEATSELEPH	ITKUYMLAVR	300
P2YR_CHICK	251	-LE--PAYCE	TGGLPRAKRK	SVETIALVLA	VEALCELEPH	VERHLYYSFR	300
	251	IYKD-LDNSP	---L-PRK--	SIYLVITVLT	VEAVSMEPH	VMTNENLRAR	300
75+13, CODING	301	310	320	330	340	350	
P2UR_MOUSE	301	STP---GVSC	FVLETFAAAY	KGIRPFASVN	SVLDEILFYF	TOCKERROPH	350
P2YR_CHICK	301	SIC-----LSC	HTENAINMAY	KTIRPEASAN	SCIDEVLYTE	AGORLVRFAR	350
	301	-LDFQTPQMC	AFNDKVYATY	QVIRGLASLN	SCVDEILYFE	AGDTERRRLS	350
75+13, CODING	351	360	370	380	390	400	
P2UR_MOUSE	351	ELLQRLTAKW	QRORV*....	400
P2YR_CHICK	351	DAKPPTEPTP	SECARRKGL	HRPNRTVRKD	LSVSSDDSR	TESTPAGSET	400
	351	RATRKSSRRS	EBNVQSKSEE	MTLNILLTEYK	QNGDTSL...	400
75+13, CODING	401	410	420	430	440	450	
P2UR_MOUSE	401	KDIRL.....	450
P2YR_CHICK	401	450

	9			18			27			36			45			54		
5'	GCC	ACC	AAC	GTG	TTC	ATC	CTG	TCA	CTG	GCC	GAT	GTG	CTG	GTG	ACA	GCC	ATC	TGC
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	Ala	Asp	Val	Leu	Val	Thr	Ala	Ile	Cys									
	63			72			81			90			99			108		
	CTG	CCG	GCC	AGT	CTG	CTG	GTA	GAC	ATC	ACG	GAA	TCC	TGG	CTC	TTT	GGC	CAT	GCC
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	Leu	Pro	Ala	Ser	Leu	Leu	Val	Asp	Ile	Thr	Glu	Ser	Trp	Leu	Phe	Gly	His	Ala
	117			126			135			144			153			162		
	CTC	TGC	AAG	GTC	ATC	CCC	TAT	CTA	CAG	GCC	GTG	TCC	GTG	TCA	GTG	GTC	GTG	CTG
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	Leu	Cys	Lys	Val	Ile	Pro	Tyr	Leu	Gln	Ala	Val	Ser	Val	Ser	Val	Val	Val	Leu
	171			180			189			198			207			216		
	ACT	CTC	AGC	TCC	ATC	GCC	CTG	GAC	CGC	TGG	TAC	GCC	ATC	TGC	CAC	CCG	CTG	TTG
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	Thr	Leu	Ser	Ser	Ile	Ala	Leu	Asp	Arg	Trp	Tyr	Ala	Ile	Cys	His	Pro	Leu	Leu
	225			234			243			252			261			270		
	TTC	AAG	AGC	ACT	GCC	CGG	CGC	GCC	CGC	GGC	TCC	ATC	CTC	GGC	ATC	TGG	GCG	GTG
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	Phe	Lys	Ser	Thr	Ala	Arg	Arg	Ala	Arg	Gly	Ser	Ile	Leu	Gly	Ile	Trp	Ala	Val
	279			288			297			306			315			324		
	TCG	CTG	GCT	GTC	ATG	GTG	CCT	CAG	GCT	GCT	GTC	ATG	GAG	TGT	AGC	AGC	GTG	CTG
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	Ser	Leu	Ala	Val	Met	Val	Pro	Gln	Ala	Ala	Val	Met	Glu	Cys	Ser	Ser	Val	Leu
	333			342			351			360			369			378		
	CCC	GAG	CTG	GCC	AAC	CGC	ACC	CGC	CTC	CTG	TCT	GTC	TGT	GAT	GAG	CGC	TGG	GCA
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	Pro	Glu	Leu	Ala	Asn	Arg	Thr	Arg	Leu	Leu	Ser	Val	Cys	Asp	Glu	Arg	Trp	Ala
	387			396			405			414			423			432		
	GAC	GAC	CTG	TAC	CCC	AAG	ATC	TAC	CAC	AGC	TGC	TTC	TTC	ATT	GTC	ACC	TAC	CTG
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	Asp	Asp	Leu	Tyr	Pro	Lys	Ile	Tyr	His	Ser	Cys	Phe	Phe	Ile	Val	Thr	Tyr	Leu
	441			450			459			468			477			486		
	GCC	CCA	CTG	GGC	CTC	ATG	GCC	ATG	GCC	TAT	TTC	CAG	ATC	TTC	CGC	AAG	CTC	TGG
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	Ala	Pro	Leu	Gly	Leu	Met	Ala	Met	Ala	Tyr	Phe	Gln	Ile	Phe	Arg	Lys	Leu	Trp
	495			504			513			522			531			540		
	GGC	CGC	CAG	ATC	CCC	GGC	ACC	ACC	TCG	GCC	CTG	GTG	CGC	AAC	TGG	AAG	CGG	CCC

FIGURE 73

```

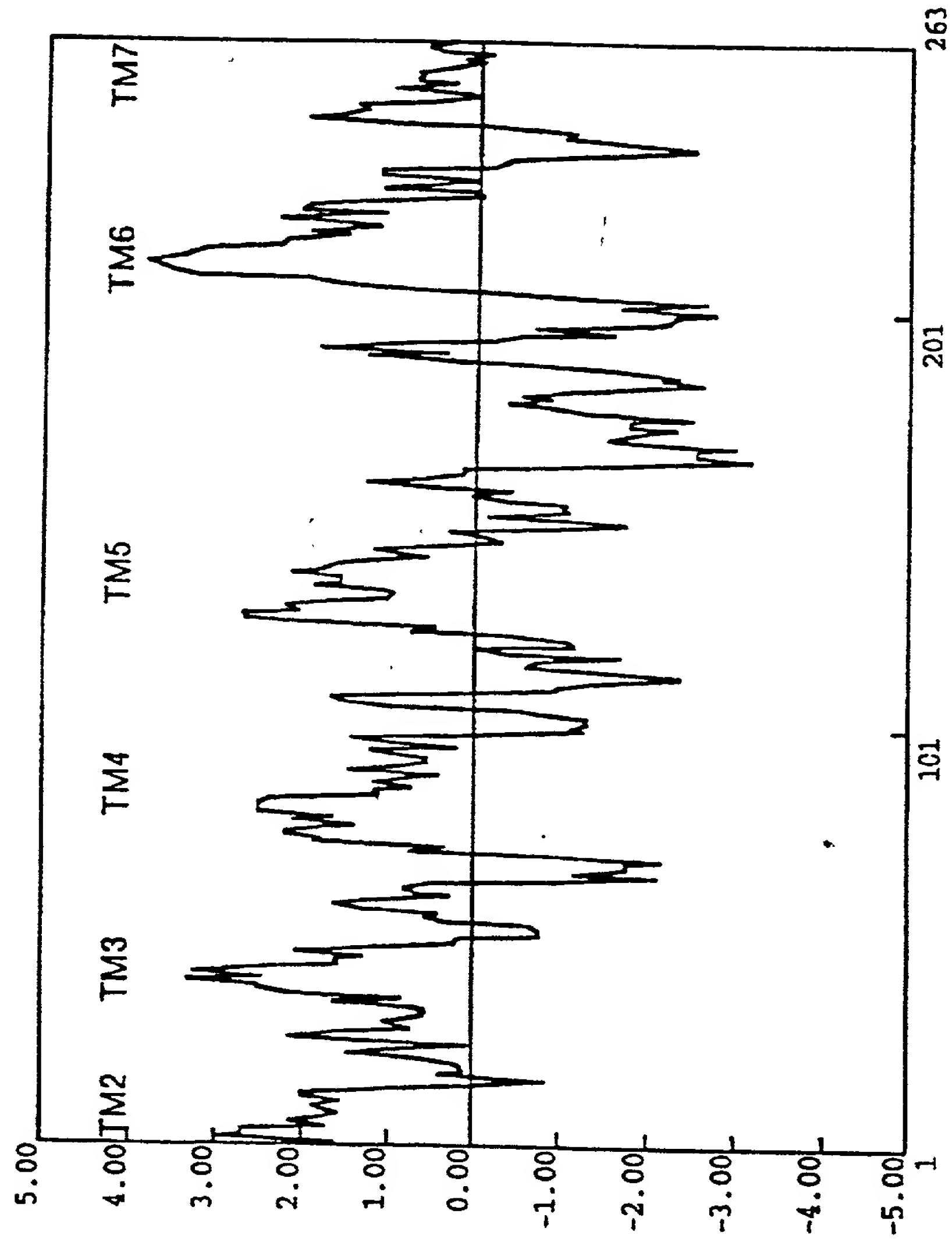
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Gly Arg Gln Ile Pro Gly Thr Thr Ser Ala Leu Val Arg Asn Trp Lys Arg Pro
      549      558      567      576      585      594
TCA GAC CAG CTG GAC GAC CAG GGC CAG GGC CTG AGC TCA GAG CCC CAG CCC CGG
-----
Ser Asp Gln Leu Asp Asp Gln Gly Gln Gly Leu Ser Ser Glu Pro Gln Pro Arg
      603      612      621      630      639      648
GCC CGC GCC TTC CTG GCC GAG GTG AAA CAG ATG CGA GCC CGG AGG AAG ACG GCC
-----
Ala Arg Ala Phe Leu Ala Glu Val Lys Gln Met Arg Ala Arg Arg Lys Thr Ala
      657      666      675      684      693      702
AAG ATG CTG ATG GTG GTG CTG CTG GTC TTC GCC CTC TGC TAC CTG CCC ATC AGT
-----
Lys Met Leu Met Val Val Leu Leu Val Phe Ala Leu Cys Tyr Leu Pro Ile Ser
      711      720      729      738      747      756
GTC CTC AAC GTC CTC AAG AGG GTC TTC GGG ATG TTC CGC CAA GCC AGC GAC CGA
-----
Val Leu Asn Val Leu Lys Arg Val Phe Gly Met Phe Arg Gln Ala Ser Asp Arg
      765      774      783      792      801      810
GAG GCC ATC TAC GCC TGC TTC ACC TTC TCC CAC TGG CTG GTG TAC GCC AAC AGC
-----
Glu Ala Ile Tyr Ala Cys Phe Thr Phe Ser His Trp Leu Val Tyr Ala Asn Ser
      819      828      837
GCC GCC AAT CCC CTC CTC TAC TCC TTC CTC CCT 3'
-----
Ala Ala

```

at the top of the page in the right margin of the page.

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FIGURE 74



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FIGURE 75

10 μ M ATP

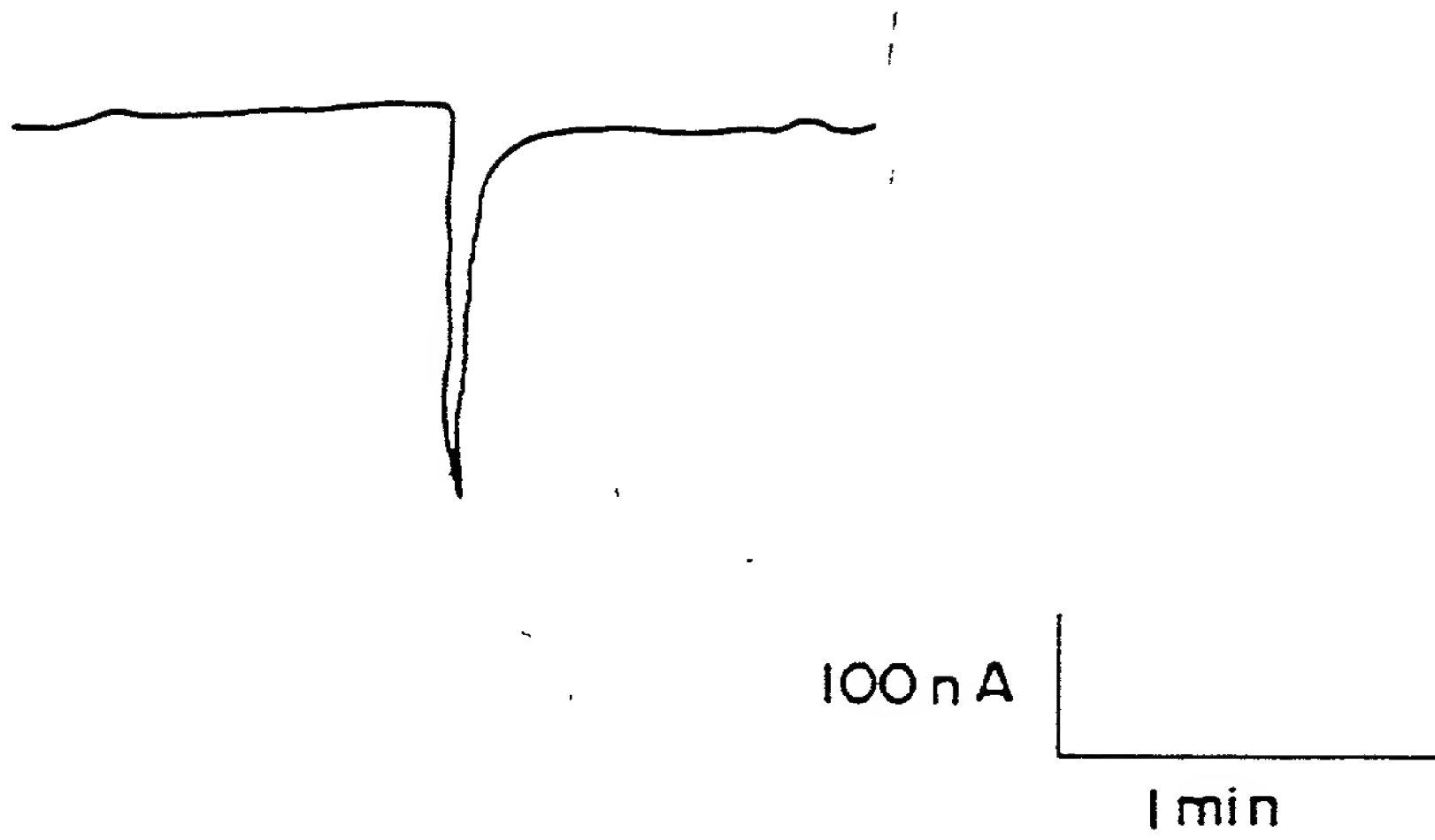


FIGURE 76

h3H2-17(5-3)	1	10	20	30	40	50	50
p3H2-17(5')	1	GTGGGCCCTGG	TGGGCAACAT	CCTGGCTTCC	TGGCACAAGC	GTGGAGGTCC	50
h3H2-17(5-3)	51	60	70	80	90	100	100
p3H2-17(5')	51	CCGTGCTGCT	TGGGTAGTGT	GTGGAGTCGT	GTGGCTGGCT	GTGACAGCCG	100
h3H2-17(5-3)	101	110	120	130	140	150	150
p3H2-17(5')	101	AGTGGCTGGC	CAAGCCATC	TTCGCTGCCA	CAGGCATCCA	CCGTAAACCG	150
h3H2-17(5-3)	151	160	170	180	190	200	200
p3H2-17(5')	151	ACTGCTGCT	ATGACCTCAG	CCGCTCTGCC	CTGGCCACCC	ACTATATGCC	200
h3H2-17(5-3)	201	210	220	230	240	250	250
p3H2-17(5')	201	CTATGGTATG	GCTCTACATG	TCAACGGCTT	CTTCTTCTCC	ATTGCTGCCC	250
h3H2-17(5-3)	251	260	270	280	290	300	300
p3H2-17(5')	251	TACAGGCTTC	CTACCTCTTC	CTGGCTCTCC	CCGCTCTCTC	CCAGGATGGC	300
h3H2-17(5-3)	301	310	320	330	340	350	350
p3H2-17(5')	301	CCAGCAGGTC	CTGTGGCCCA	AGAGCGGGCC	AGCAAGGCGG	CTCGTATGGC	350
h3H2-17(5-3)	351	360	370	380	390	400	400
p3H2-17(5')	351	TGTGGTGGTG	GCAGCTGTCT	TGGCCCTCTG	CTGGCTGCCT	CTCTAC....	400

FIGURE 77

1	TGACTCCCTGAACATAGGAAACCCACCTGGGCAGCCATGGAATGGGACAATGGCACAGGC	60
1	MetGluTrpAspAsnGlyThrGly	8
61	CAGGCTCTGGGCTTGCCACCCACCACCTGTGTCTACCGCGAGAACTTCAAGCAACTGCTG	120
8	GlnAlaLeuGlyLeuProProThrThrCysValTyrArgGluAsnPheLysGlnLeuLeu	28
121	CTGCCACCTGTGTATTTCGGCGGTGCTGGCGGCTGGCCTGCCGCTGAACATCTGTGTCATT	180
28	LeuProProValTyrSerAlaValLeuAlaAlaGlyLeuProLeuAsnIleCysValIle	48
181	ACCCAGATCTGCACGTCCCGCCGGCCCTGACCCGACGGCCGTGTACACCCTAAACCTT	240
48	ThrGlnIleCysThrSerArgArgAlaLeuThrArgThrAlaValTyrThrLeuAsnLeu	68
241	GCTCTGGCTGACCTGCTATATGCCTGCTCCCTGCCCCCTGCTCATCTACAACCTATGCCCAA	300
68	AlaLeuAlaAspLeuLeuTyrAlaCysSerLeuProLeuLeuIleTyrAsnTyrAlaGln	88
301	GGTGATCACTGGCCCTTTGGCGACTTCGCCTGCCGCTGGTCCGCTTCCTCTTCTATGCC	360
88	GlyAspHisTrpProPheGlyAspPheAlaCysArgLeuValArgPheLeuPheTyrAla	108
361	AACCTGCACGGCAGCATCCTCTTCCTCACCTGCATCAGCTTCCAGCGCTACCTGGGCATC	420
108	AsnLeuHisGlySerIleLeuPheLeuThrCysIleSerPheGlnArgTyrLeuGlyIle	128
421	TGCCACCCGCTGGCCCCCTGGCACAACCGTGGGGGCGCGGGGCTGCCTGGCTAGTGTGT	480
128	CysHisProLeuAlaProTrpHisLysArgGlyGlyArgArgAlaAlaTrpLeuValCys	148
481	GTAACCGTGTGGCTGGCCGTGACAACCCAGTGCCTGCCCCACAGCCATCTTCGCTGCCACA	540
148	ValThrValTrpLeuAlaValThrThrGlnCysLeuProThrAlaIlePheAlaAlaThr	168
541	GGCATCCAGCGTAACCGCACTGTCTGCTATGACCTCAGCCCGCCTGCCCTGGCCACCCAC	600
168	GlyIleGlnArgAsnArgThrValCysTyrAspLeuSerProProAlaLeuAlaThrHis	188
601	TATATGCCCTATGGCATGGCTCTCACTGTCTATCGGCTTCCTGCTGCCCTTTGCTGCCCTG	660
188	TyrMetProTyrGlyMetAlaLeuThrValIleGlyPheLeuLeuProPheAlaAlaLeu	208
661	CTGGCCTGCTACTGTCTCCTGGCCTGCCGCTGTGCCGCCAGGATGGCCCGGCAGAGCCT	720
208	LeuAlaCysTyrCysLeuLeuAlaCysArgLeuCysArgGlnAspGlyProAlaGluPro	228
721	GTGGCCCAGGAGCGGCGTGGCAAGGCGGCCGCATGGCCGTGGTGGTGGCTGCTGCCCTTT	780
228	ValAlaGlnGluArgArgGlyLysAlaAlaArgMetAlaValValAlaAlaAlaPhe	248
781	GCCATCAGCTTCCTGCCTTTTCACATCACCAAGACAGCCTACCTGCCAGTGGGCTCGACG	840
248	AlaIleSerPheLeuProPheHisIleThrLysThrAlaTyrLeuAlaValGlySerThr	268
841	CCGGGCGTCCCCTGCCTGTATTGGAGGCCTTTGCAGCGGCCTACAAAGGCACGCGGCCG	900
268	ProGlyValProCysThrValLeuGluAlaPheAlaAlaAlaTyrLysGlyThrArgPro	288
901	TTTGCCAGTGCCAACAGCGTGTGGACCCCATCCTCTTCTACTTCACCCAGAAGAAGTTC	960
288	PheAlaSerAlaAsnSerValLeuAspProIleLeuPheTyrPheThrGlnLysLysPhe	308
961	CGCCGGCGACCATGAGCTCTACAGAACTCACAGCCAAATGGCAGAGGCAGGGTCGC	1020
308	ArgArgArgProHisGluLeuLeuGlnLysLeuThrAlaLysTrpGlnArgGlnGlyArg	328
1021	TGA	1023
328	***	329

FIGURE 78

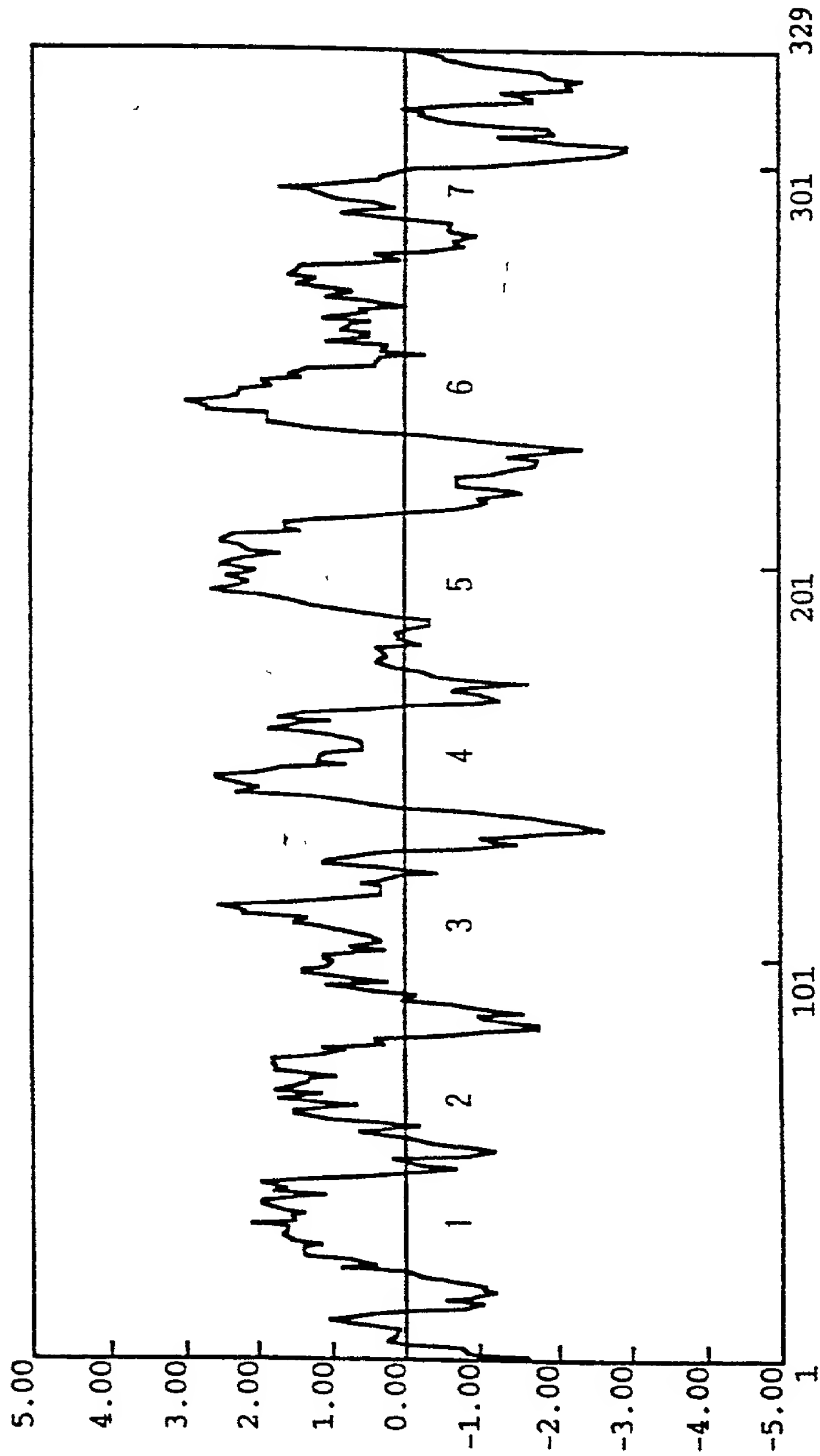


FIGURE 79

human prino, mouseFULL3H2	1	MEWDNGTGOA	10	LGLPPTTCVY	20	RENFKQLLLP	30	PVYSVLAAG	40	LPLNICVITQ	50
	1	MEQDNGTGOA		PGLPPTTCVY		REDEKQLLLT		PVYSVLAAG		LPLNICVITQ	50
human prino, mouseFULL3H2	51	ICPSRRALTR	60	TAVYTLNLAL	70	ADLLYACSLP	80	LLIYNVACGD	90	HWPFQDFACR	100
	51	ICASRRFLTR		SAVYTLNLAL		ADLLYACSLP		LLIYNVACGD		HWPFQDFACR	100
human prino, mouseFULL3H2	101	LVRFLFYANL	110	HGSILFLTCT	120	SFQRYLGICH	130	PLASWHKRGG	140	RRRAAMVVCVT	150
	101	FVRFLFYANL		HGSILFLTCT		SFQRYLGICH		PLASWHKRGG		RRRAAMVVCVT	150
human prino, mouseFULL3H2	151	VMLAVTQCL	160	PTAVFAATGI	170	QRNRTVCYDL	180	SPPALATHYM	190	PYGMALIVIG	200
	151	VMLAVTQCL		PTAVFAATGI		QRNRTVCYDL		SPPALATHYM		PYGMALIVIG	200
human prino, mouseFULL3H2	201	ELLPFALLA	210	CYCLACRLC	220	RODGPABEVA	230	QERRSKAARM	240	AVVWAAAFAT	250
	201	ELLPFALLA		CYCLACRLC		RODGPABEVA		QERRSKAARM		AVVWAAAFAT	250
human prino, mouseFULL3H2	251	SFLPFHITKT	260	AYLAVGSTPG	270	VPCTVLEAFA	280	AAKKGTRPFA	290	SANSVLDPIL	300
	251	SFLPFHITKT		AYLAVGSTPG		VPCTVLEAFA		AAKKGTRPFA		SANSVLDPIL	300
human prino, mouseFULL3H2	301	FYFTQKKFRF	310	RPHLLQKLT	320	AKMQRQR*	330	340	350
	301	FYFTQKKFRF		RPHLLQKLT		AKMQRQR*		350

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DIKE, BRONSTEIN, ROBERTS & CUSHMAN, LLP
130 Water Street
Boston, Massachusetts 02109

Attorney's Docket No. 45753-DIV2**DECLARATION AND POWER OF ATTORNEY**

As a below named inventor, I hereby declare that: My residence, post office address and citizenship are as stated below next to my name. I believe I am the original, first and sole inventor (if only one name is listed at 201) below or an original, first and joint inventor (if plural names are listed at 201-203 below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

G PROTEIN COUPLED RECEPTOR PROTEIN PRODUCTION, AND USE THEREOF

which is described and claimed in:

- ☒ the specification attached hereto.
- ☐ the specification in U.S. Application Serial Number , _____
 filed on _____.
- ☐ the specification in PCT international application Number _____,
 filed on _____; and was amended on _____.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a). I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

Prior Foreign/PCT Applications and Any Priority Claims Under 35 U.S.C. 119:			
Application No.	Filing Date	Country	Priority Claimed Under 35 U.S.C. 119?
6-189272	8/11/94	Japan	X YES <input type="checkbox"/> NO
6-189273	8/11/94	Japan	X YES <input type="checkbox"/> NO
6-189274	8/11/94	Japan	X YES <input type="checkbox"/> NO
6-236356	9/30/94	Japan	X YES <input type="checkbox"/> NO
6-236357	9/30/94	Japan	X YES <input type="checkbox"/> NO
6-270017	11/02/94	Japan	X YES <input type="checkbox"/> NO
6-326611	12/28/94	Japan	X YES <input type="checkbox"/> NO

- 2 -

Prior Foreign/PCT Applications and Any Priority Claims Under 35 U.S.C. 119:			
7-007177	1/20/95	Japan	X YES <input type="checkbox"/> NO
7-057186	3/16/95	Japan	X YES <input type="checkbox"/> NO
7-093989	4/19/95	Japan	X YES <input type="checkbox"/> NO
7-074314	3/31/95	Japan	X YES <input type="checkbox"/> NO

I hereby claim the benefit under 35 U.S.C. §120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below, and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose material information as defined in 37 CFR §1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

Prior U.S. Applications or PCT International Applications Designating the U.S.-Benefit Under 35 U.S.C. §120					
U.S. Applications		Status (Check One)			
Application Serial No.	U.S. Filing Date	Patented	Pending	Abandoned	
08/513,974	9/14/95		X		
09/038,572	3/11/98		X		
PCT Applications Designating the U.S.					
Application No.	Filing Date	U.S. Serial No. Assigned			
PCT/JP95/01599	8/10/95	08/513,974		X	

CLAIM FOR BENEFIT OF PRIOR U.S. PROVISIONAL APPLICATION(S)
(35 U.S.C. § 119(e))

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below:

Applicant	Provisional Application Number	Filing Date

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) with full powers of association, substitution and revocation to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

- 3 -

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William J. Daley, Jr.	(Reg. No. 35,487)	Linda M. Buckley	(Reg. No. 31,003)
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I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Signature of Inventor 201

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Date:

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